Volume 20 | Supplement 6 | November 2018

Neuro-Oncology

Official Journal of the Society for Neuro-Oncology since 1997

Abstracts from the 23rd Annual Scientific Meeting and Education Day of the Society for Neuro-Oncology
November 15 – 18, 2018
New Orleans, Louisiana

Including
Abstracts from the 3rd CNS Anticancer Drug Discovery and Development Conference
November 14 – 15, 2018
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Mission statement: The goal of *Neuro-Oncology* is to provide the international neuro-oncology community with high-quality, rapid publication of information in all fields. The journal welcomes submissions from throughout the world and encourages the participation of neuro-oncologists in a wide array of subspecialities. Areas covered include, but are not limited to, biochemistry and biophysics, clinical neuro-oncology, clincial therapy trials, epidemiology, imaging, immunology, medical neuro-oncology, neuropathology, molecular genetics, pediatric neuro-oncology, preclinical experimental therapeutics, retrospective clinical studies, surgical neuro-oncology, and tumor biology. Published by Oxford University Press, *Neuro-Oncology* is the official journal for the Society for Neuro-Oncology (SNO), the Japan Society for Neuro-Oncology, the European Association for Neuro-Oncology, and the World Federation of Neuro-Oncology Societies.

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Abstracts

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ACTR - Adult Clinical Trials – Non-immunologic
ANGI - Angiogenesis and Invasion
ATIM - Adult Clinical Trials – Immunologic
CBMT - Cell Biology and Metabolism
CMET - CNS Metastasis
COMP - Computational Omics
CSIG - Cell Signaling and Signaling Pathways
DDIS - Drug Discovery
DRES - Drug Resistance
EPID - Epidemiology
EXTH - Experimental Therapeutics
GENE - Genetics and Epigenetics
HOUT - Health Outcome Measures
IMMU - Immunology
INNV - Innovations in Patient Care
LTBK - Late-Breaking
MNGI - Meningioma
NCMP - Neurological Complications of Cancer and Cancer Therapy
NCOG - Neuro-Cognitive Outcomes
NIMG - Neuro-Imaging
PATH - Molecular Pathology and Classification – Adult and Pediatric
PDCT - Pediatric Clinical Trials
PDTM - Pediatric Tumors
QOLP - Quality of Life and Palliative Care
RARE - Rare Tumors
RBTT - Randomized Brain Tumor Trials in Development
RDNA - Radiation Biology and DNA Repair
RTHP - Radiation Therapy
STEM - Stem Cells
SURG - Surgical Therapy
TMIC - Tumor Microenvironment
TMOD - Tumor Models
CADD - CNS Anticancer Drug Discovery and Develpment

ADULT CLINICAL TRIALS – IMMUNOLOGIC

ATIM-02. TUMOR TREATING FIELDS IN COMBINATION WITH BEVACIZUMAB IN RECURRENT OR PROGRESSIVE MENINGIOMA IN A PHASE 2 STUDY

Priya Kumthekar, Tim Kruser, Sean Sachev, Jeffrey Raizer, Sean Grimm and Karen Dixit; Northwestern University, Chicago, IL, USA

For patients with WHO grade II meningioma, the two-year and five-year overall survival is 93% and 73%, respectively. Patients with malignant meningioma (grade III) have significantly worse outcomes, with five-year overall survival rates of 42%. The recurrence rate remains high with overall survival is as low as 3 years after diagnosis. The current treatment for high grade and recurrent meningioma includes maximal safe surgical resection and radiation. Tumor Treating Fields (TTFields) and Bevacizumab (BEV) have been reported to potentially show activity in recurrent and high-grade meningioma. The combination of TTFields and BEV may provide a viable treatment option for patients with recurrent and high grade meningioma. This Phase 2 Simon two-staged, non-randomized, open-label phase II, single arm clinical trial [NCT02847559] will investigate the combination of TTFields and BEV in recurrent or progressive meningioma. The primary endpoint is progression free survival (PFS) at 6 months (PFS-6). Secondary endpoints include overall survival (OS); Tumor Response Rate (TRR), Objective Response Rate (ORR); and Quality of Life (QOL) using FACT-Br questionnaire. Patients (N=27), including those who have failed prior BEV therapy, will receive BEV IV 10 mg/kg over 30–90 minutes on days 1 and 15 of courses 1–4. Beginning on day 1 of course 5, patients may choose to receive bevacizumab IV 15 mg/kg every 3 weeks or remain on the every 2-week schedule. Patients will receive TTFields 200 KHz (1–3 V/cm) daily for at least 18 hours/day. This study is powered to detect a true PFS-6 rate of 20% with 80% probability (80% power) and a true PFS-6 rate of 5% with 90% probability (alpha=0.10). 27 patients were planned to have 24 evaluable patients. To-date 4 patients have been enrolled at the principal site and at least two additional sites are planned to open this year.

ATIM-03. TTFIELDS AND PULSED BEVACIZUMAB IN PATIENTS WITH BEVACIZUMAB-REFRACTORY RECURRENT GLIOBLASTOMA: A PHASE 2 STUDY

David Tran[1], Ashley Ghiaseddin[1], Jian Campian[2], Stephen Staal[1], Sonisha Warren[1], Anne Allen[1], Deborah Sampson[1], Valerie Greene[1] and George Ansstas[2]; [1]University of Florida, Gainesville, FL, USA, [2]Washington University, St. Louis, MO, USA

Despite aggressive treatment with surgery, radiation therapy and chemotherapy, the median overall survival for recurrent glioblastoma (rGBM) averages 25 weeks. Our prior experience in 8 bevacizumab-refractory GBM patients initially treated with TTFields monotherapy and re-challenged with bevacizumab on progression suggest that successive cycles of on/off (or pulsed) bevacizumab dosing will produce peaks and troughs in the mitotic activities of glioma cells that render these cells more sensitive to the antimitotic activity of TTFields during peak growth rates and may lower disease burden and increase survival. This Phase 2, single arm, open label study [NCT02663271] investigates if TTFields combined with pulsed bevacizumab increases overall survival in bevacizumab-refractory GBM compared to historical controls treated with continuous bevacizumab alone or in combination with standard chemotherapy. Twenty-five adults male or female patients with bevacizumab-refractory rGBM (WHO grade IV) aged 22 years, KPS>60 will undergo 12 months of planned continuous TTFields (200 KHz) (60–75% compliance goal; patients<60% compliance at Month 2 withdrawn) followed by pulsed bevacizumab (10 mg/kg IV/ 2 weeks) on further progression (RANO), with option of extending treatment to 24 months in patients not progressed and/or have adequate performance status at the 12-month mark. Pulsed bevacizumab dosing is defined by at least one cycle on and at least one cycle off. A cycle is defined as 8 weeks in length. Primary endpoint is overall survival between the groups. Secondary endpoints include adverse events, KPS, QoL (Mini-Mental Status Exam) and response rate (RANO). Largest hazard ratio (HR)<1 (or smallest median survival time >3.3 months) that can be detected at 80%, 90%, or 95% power and a 1-tailed significance level of 0.05, by sample size (N=20 to 36, or 10 to 18 months of accrual at 2 patients per month) and shape parameter k=1.50, 2.50. To date 3 patients have been enrolled.

ATIM-05. INTRATUMORAL DELIVERY OF MDNA55, AN INTERLEUKIN-4 RECEPTOR TARGETED IMMUNOTHERAPY, BY MRI-GUIDED CONVECTIVE DELIVERY FOR THE TREATMENT OF RECURRENT GLIOBLASTOMA

Achal Achrol[1], Martin Bexon[2], Krystof Bankiewicz[3], Andrew J. Brenner[4], Nicholas Butowski[5], Santosh Kesari[1], Fahar Merchant[2], Rosemina Merchant[2], Dina Randazzo[6], Michael Vogelbaum[7], Miroslaw Zabek[8] and John Sampson[9]; [1]John Wayne Cancer Institute and Pacific Neuroscience Institute, Santa Monica, CA, USA, [2]Medicenna Biopharma, Houston, TX, USA, [3]Department of Neurological Surgery, University of California San Francisco, San Francisco, CA, USA, [4]Mays Cancer Center/ UT Health San Antonio, San Antonio, TX, USA, [5]University of California, San Francisco, San Francisco, CA, USA, [6]The Preston Robert Tisch Brain Tumor Center, Duke University Medical Center, Durham, NC, USA, [7]Department of Neurosurgery, Cleveland Clinic, Cleveland, OH, USA, [8]Mazovian Brodnowski Hospital, Warsaw, Mazowieckie, Poland, [9]Duke University Medical Center, Durham, NC, USA

While intratumoral delivery of immunotherapies allows for targeted distribution and minimal systemic toxicity, optimal spatial distribution of therapeutic agents remains a challenge. MR-guided convection-enhanced delivery of MDNA55, interleukin-4 cytokine fused to Pseudomonas exotoxin, is currently being explored in a Phase 2 open label study in up to 52 patients with recurrent glioblastoma. MDNA55 is coinfused with a Gadolinium-based contrast agent (GdDTPA) and delivered as a single infusion using implantable flexible catheters. MRI was employed to monitor initial infusion, allowing adjustment of catheter depth as needed. The bulk of the delivery was performed outside the MRI scanner while patients were awake. MRI confirmation of distribution was performed within 4 hours post-infusion. Here we report preliminary results of tumor distribution in 23 subjects. Total volume of MDNA55 (ranging from 12 mL to 66 mL) was initially administered to subjects (n=12) based on tumor size. This achieved a mean coverage of 75% (range 46 – 94%) with 17% showing volume of distribution > 100 mL. Review by the Safety Committee determined that improvements to drug distribution could be further enhanced by moving to a fixed volume of 60 mL administered via 4 catheters and allowing placement outside the peritumoral area if necessary. Treatment in the next 11 subjects showed that infusion volumes exceeding 40 mL and placement of catheters outside the enhancing tumor led to increased volumes of drug distribution (45% showed volume of distribution > 100 mL) but did not improve the

target percentage coverage of the tumor (mean 60%, range 22 – 97%) and its immediate penumbra. Therefore, under the current protocol version, all subjects are receiving individualized volume of MDNA55 (according to tumor size), but not exceeding 40 mL. Further optimization is underway to ensure adequate coverage and improve drug distribution.

ATIM-06. PHASE 2 TRIAL OF SL-701 + BEVACIZUMAB IN PATIENTS WITH PREVIOUSLY TREATED GLIOBLASTOMA (GBM) MEETS PRIMARY ENDPOINT OF OS-12, WITH PRELIMINARY CORRELATION BETWEEN LONG-TERM SURVIVAL AND TARGET-SPECIFIC CD8+ T CELL IMMUNE RESPONSE

David Peereboom[1], L. Burt Nabors[2], Priya Kumthekar[3], Michael Badruddoja[4], Karen Fink[5], Frank Lieberman[6], Surasak Phuphanich[7], Erin Dunbar[8], Tobias Walbert[9], David Schiff[10], David Tran[11], Lynn Ashby[12], Nicholas Butowski[13], Fabio Iwamoto[14], Ross Lindsay[15], John Bullington[15], Michael Schulder[16], Jonathan Sherman[17], Chris Brooks[15] and David Reardon[18]; [1]Cleveland Clinic, Cleveland, OH, USA, [2]University of Alabama at Birmingham, Birmingham, AL, USA, [3]Northwestern University, Chicago, IL, USA, [4]Center for Neurosciences, Tucson, AZ, USA, [5]Baylor University, Dallas, TX, USA, [6]University of Pittsburgh, Pittsburgh, PA, USA, [7]Cedars Sinai Medical Center, Los Angeles, CA, USA, [8]Piedmont Brain Tumor Center, Atlanta, GA, USA, [9]Henry Ford Hospital, Detroit, MI, Detroit, MI, USA, [10]University of Virginia, Charlottesville, VA, USA, [11]University of Florida, Gainesville, FL, USA, [12]St. Joseph's Hospital and Medical Center, Phoenix, AZ, USA, [13]UC San Francisco, San Francisco, CA, USA, [14]Department of Neurology and Herbert Irving Comprehensive Cancer Center, Columbia University Irving Medical Center, New York, NY, USA, [15]Stemline Therapeutics, New York, NY, USA, [16]North Shore University Hospital, Manhasset, NY, USA, [17]George Washington University, Washington, DC, USA, [18]Dana-Farber Cancer Institute, Boston, MA, USA

SL-701 is a novel immunotherapy comprised of synthetic peptides designed to elicit an anti-tumor immune response against GBM targets: interleukin-13 receptor alpha-2, EphrinA2 and Survivin. Updated Phase 2 data are reported. Patients with previously treated GBM, bevacizumab (bev)-naive, HLA-A2+, and KPS>60, were enrolled. SL-701 with adjuvant poly-ICLC was dosed biweekly with bev (10 mg/kg) for 6 months, then q28 days. Primary endpoint was OS-12, with statistical significance determined if lower bound of 2-sided 95% Clopper Exact confidence interval (CI) is >20%. Target-specific CD8+ T-cell frequency was assessed by flow cytometry. 28 patients received median of 13 SL-701 doses with bev. Most frequent treatment-related adverse events (TRAEs) were fatigue (39%) and injection site reaction (25%). Grade 3 TRAE was fatigue (3.6%); there were no other grade 3 TRAEs. Disease control rate (complete response [CR], partial response [PR], stable disease) was 54% (n=15), with 2 CRs and 2 PRs. Median duration of disease control was 8.8 months (95% CI 3.7, NE). Median OS was 11.7 months (95% CI: 7.1, NE). The primary endpoint was met with a 50% OS-12 (95% CI: 30.6, 69.4). Target-specific CD8+ T cell activity was detected at 8–24 wks, the majority occurring by wk 16. Long-term survivors were largely comprised of patients with target-specific CD8+ T-cell responses; median OS of these immune responders was not reached. The Phase 2 trial of SL-701 + bev in previously treated GBM met its primary endpoint with a 50% OS-12. The regimen was well-tolerated and demonstrated objective responses, including CRs. There was an even more pronounced survival signal, as well as an encouraging survival tail comprised largely of target-specific CD8+ T cell responders, and the median OS of these immune responders was not reached. Updates around next steps, including leveraging potential immune-related biomarkers in a registration-directed trial design, will be provided.

ATIM-07. WINDOW-OF-OPPORTUNITY CLINICAL TRIAL OF PEMBROLIZUMAB IN RECURRENT GLIOBLASTOMA PATIENTS

John de Groot[1], Marta Penas-Prado[2], Jacob Mandel[3], Barbara O'Brien[2], Shiao-Pei Weathers[2], Monica Loghin[2], Carlos Kamiya-Matsuoka[2], Shouhao Zhou[2], Rivka Colen[4], Kathy Hunter[2], Gregory Fuller[2], Jason T Huse[2], Ganesh Rao[2], Jeffrey Weinberg[2], Sujit Prabhu[2], Sherise Ferguson[2], Ying Yuan[2], Luis Vence[2], James Allison[2], Padmanee Sharma[2] and Amy Heimberger[2]; [1]Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [2]University of Texas MD Anderson Cancer Center, Houston, TX, USA, [3]Baylor College of Medicine, Houston, TX, USA, [4]Department of Cancer Systems Imaging, UT MD Anderson Cancer Center, Houston, TX, USA

BACKGROUND: A window-of-opportunity trial was used to ascertain the immune modulatory properties of pembrolizumab in recurrent GB patients. METHODS: In an open label, single-center, single-arm Phase 2 trial, 15 patients with recurrent GBM, were treated with up to two doses of pembrolizumab prior to surgery and afterwards received pembrolizumab until disease progression or the development of unacceptable toxicities. The co-primary objectives of the study were to evaluate immune effector function in resected GBM tissue and to determine progression free survival at 6 months (PFS6). RESULTS: We screened 21 patients, of whom 15 were enrolled and received at least one dose of pembrolizumab. The most common adverse event was grade 1 or 2 fatigue in 40% of patients followed by headache in 27%. There were five grade 3 or 4 adverse events including one with cerebral edema requiring surgery. There were no treatment-related deaths; 5 (33%) of 15 patients died because of progression of disease. The median follow-up time for all patients was 12 months (95% CI3-31). Based on iRANO, 10 patients had progressive disease, 3 had a partial response and 1 had stable disease. The longest ongoing duration of response exceeded 34 months in two patients. Median PFS was 7 months (95% CI 4–16) and PFS6 was 53% (95% CI 33%-86%). Median OS was not reached (95% CI 15 to not reached), with an estimated 1-year overall survival of 72% (52%–99.6%). CyTOF and IHC immune analysis revealed that GBM tumors were markedly enriched with CD68+ macrophages but had a paucity of effector T cells. CONCLUSIONS: Although pembrolizumab was well tolerated, a high predominance of immunosuppressive CD68+ myeloid cells and a marked scarcity of T cells within the tumor microenvironment may be limiting the activity of PD-1 blockade.

ATIM-08. A PHASE I TRIAL OF PEMBROLIZUMAB AND VORINOSTAT COMBINED WITH TEMOZOLOMIDE AND RADIATION THERAPY FOR NEWLY DIAGNOSED GLIOBLASTOMA (NCT03426891)

Solmaz Sahebjam, Peter Forsyth, Nam Tran, Sepideh Mokhtari, John Arrington, Michael Jaglal, Arnold Etame, James Liu, Melissa Wicklund, Tyra Gatewood, Robert Macaulay, Timothy Robinson and Michael Yu; H. Lee Moffitt Cancer Center, Tampa, FL, USA

BACKGROUND: A growing body of evidence indicates that epigenetic silencing of genes involved in antigen processing and immune recognition results in immune escape and resistance to immunotherapy. Pre-clinical experiments have shown that use of histone deacetylases inhibitors such as vorinostat can restore tumor immune recognition and synergize with anti-PD 1/PD-L1 antibodies. Moreover, vorinostat has radiosensitizing properties. This report describes an ongoing phase I trial of vorinostat in combination with an antibody against PD1 (pembrolizumab), radiotherapy and temozolomide (TMZ) in patients (pts) with newly diagnosed glioblastoma (GBM). METHOD: This study employs a standard 3 + 3 dose escalation design exploring 2 sequential dose escalation cohorts of vorinostat. Eligible pts are treated with concurrent radiotherapy (60 Gy in 30 fractions) with TMZ (75 mg/m^2/day) followed by 6 cycles of maintenance TMZ. Pembrolizumab (200 mg) is administered intravenously once every 3 weeks. Dose level 1 is consistent of vorinostat 200 mg/day orally on days 1–5 every week during radiotherapy and 300 mg/day orally 1 week on 1 week off after radiotherapy. At dose level 2, pts receive 300 mg of vorinostat on days 1–5 every week during radiotherapy and 400 mg/day orally 1 week on 1 week off after radiotherapy. Once the recommended phase II dose (RP2D) of vorinostat is determined, an additional 20 pts will be enrolled in an expansion safety cohort. The primary study objectives are to determine safety and the RP2D of vorinostat administered with above combination in pts with GBM. Secondary endpoints include determination of the 12 and 24 month survival rates and exploring tissue and blood biomarkers. RESULTS: As of June 4, 2018, enrollment to dose level 1 has been completed. So far no does limiting adverse event has been observed. Thrombocytopenia and fatigue are the most common adverse events. Updated safety and efficacy results will be presented.

ATIM-10. A PHASE I/II CLINICAL TRIAL OF AUTOLOGOUS CMV-SPECIFIC CYTOTOXIC T CELLS (CMV-TC) FOR GLIOBLASTOMA: DOSE ESCALATION AND CORRELATIVE RESULTS

Marta Penas-Prado[1], Shiao-Pei Weathers[1], Shouhao Zhou[1], Carlos Kamiya-Matsuoka[1], Barbara O'Brien[1], Monica Loghin[1], Rebecca Harrison[1], Be Lian Pei[1], Sandra Ictech[1], Kathy Hunter[1], W.K. Alfred Yung[2], John de Groot[2], Elizabeth J Shpall[1], Amy Heimberger[1] and Katy Rezvani[1]; [1]University of Texas MD Anderson Cancer Center, Houston, TX, USA, Houston, TX, USA, [2]Department of Neuro-Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA

BACKGROUND: Cytomegalovirus (CMV) antigens are present in > 90% of GBMs but not in normal brain. CMV-TC in GBM tumor tissue have their effector function suppressed. Highly functional CMV pp65 specific T cells can be expanded *in vitro* from peripheral blood (PB) of GBM patients. METHODS: Autologous polyclonal CD8+ and CD4+ CMV-TC from patients with recurrent GBM were expanded *ex vivo* under GMP-compliant conditions and administered after 3 weeks of lymphodepleting dose-dense temozolomide (ddTMZ, 100 mg/m2); 4 dose levels (5 x 10^6 cells to 1 x 10^8 cells), 3 + 3 design. Treatment was repeated q 6 weeks; total of 4 cycles. Eligibility: ≥18 years of age, KPS ≥60, CMV sero+, on ≤ 2 mg of dexamethasone daily, any number of relapses. Imaging response evaluated by MRI q 6 weeks. *In vivo* persistence and expansion of adoptively-infused CMV-TC determined by dextramer staining and multiparameter flow cytometry in serially-sampled PB. RESULTS: 34 patients screened, 18 underwent leuka-

pheresis, 15 completed cycle 1. Median age 56 (27–69), median KPS 90; 11 were at 1st, 3 at 2nd and 1 at 3rd relapse. MGMT methylated in 6, unmethylated in 3, indeterminate/unknown in 6. IDH status wildtype in 10, mutated in 3, unknown in 2. No dose limiting toxicities (DLTs) observed. Complete radiographic response observed in 1 patient, partial response in 2, stable disease in 6, and progressive disease in 6. Repeated infusions of CMV-TC were associated with significant increase in circulating CMV+ CD8+ T-cells, but cytokine production (CD107a, TNFα, IFNγ, IL2) was suppressed (dose level 4 analysis ongoing). CONCLUSIONS: Adoptive infusion of CMV-TC after lymphodepleting therapy with ddTMZ was well tolerated with no DLTs; 1 x 10e8 confirmed as safe dose. Effector function in PB was suppressed. Correlative studies of CMV-specific T cell effector function in tumor microenvironment will be assessed in window-of-opportunity expansion cohort.

ATIM-12. NEOADJUVANT ANTI-PD-1 IMMUNOTHERAPY PROMOTES INTRATUMORAL AND SYSTEMIC IMMUNE RESPONSES IN RECURRENT GLIOBLASTOMA: AN IVY CONSORTIUM TRIAL

Robert Prins[1], Aaron Mochizuki[2], Joey Orpilla[3], Alexander Lee[4], Tom Davidson[5], Sarah Gaffey[6], Catherine Sanders[7], Julie Rytlewski[7], Benjamin Ellingson[8], Gang Li[5], William Yong[9], Jennifer Clarke[10], Isabel Arrillaga-Romany[11], Howard Colman[12], David Reardon[13], Thomas Kaley[14], John de Groot[15], Linda Liau[3], Patrick Wen[16] and Timothy Cloughesy[17]; [1]UCLA Depts. of Neurosurgery and Molecular and Medical Pharmacology, LA, CA, USA, [2]UCLA Pediatrics, LA, CA, USA, [3]UCLA Neurosurgery, Los Angeles, CA, USA, [4]UCLA Dept of Molecular and Medical Pharmacology, Los Angeles, CA, USA, [5]David Geffen School of Medicine, Los Angeles, CA, USA, [6]Dana Farber Cancer Institute, Boston, MA, USA, [7]Adaptive Biotechnologies, Corp., Seattle, WA, USA, [8]University of California Los Angeles, Los Angeles, CA, USA, Los Angeles, CA, USA, [9]UCLA Dept. of Pathology and Laboratory Medicine, Los Angeles, CA, USA, [10]University of California at San Francisco, San Francisco, CA, USA, [11]Neuro-Oncology, MGH Cancer Center, Boston, MA, USA, [12]Department of Neurosurgery, Huntsman Cancer Institute and Clinical Neuroscience Center, University of Utah, Salt Lake City, UT, USA, [13]Dana-Farber Cancer Institute, Boston, MA, USA, [14]Memorial Sloan Kettering Cancer Center, New York City, NY, USA, [15]Department of Neuro-Oncology, The University of Texas MD Anderson Cancer Center, Houston, TX, USA, [16]Center For Neuro-Oncology, Dana-Farber Cancer Institute, Boston, MA, USA, [17]UCLA Neuro-Oncology, Los Angeles, CA, USA

Glioblastoma is the most common malignant brain tumor in adults and is associated with poor survival. It is often resistant to standard-of-care chemotherapy and radiation, necessitating the development of more effective treatments. The Ivy Foundation Early Phase Clinical Trials' Consortium conducted a randomized, multi-institution clinical trial to evaluate the immune response and survival following neoadjuvant and adjuvant therapy with pembrolizumab, a PD-1 monoclonal antibody, in thirty patients with recurrent, surgically resectable glioblastoma. Patients who received neoadjuvant pembrolizumab, with continued adjuvant therapy following surgery, had significantly extended overall survival compared to patients that received adjuvant, post-surgical PD-1 blockade alone (HR=0.33, p<0.008, log-rank test). Survival was directly associated with an elevated IFN-g gene expression signature in the tumor, but only in patients who received neoadjuvant PD-1 blockade (HR=0.15, p<0.02, Wald test). Focal induction of PD-L1 in the tumor microenvironment was observed in the neoadjuvant group and linked with the IFN-g gene expression signature and extended survival. Similarly, neoadjuvant pembrolizumab was associated with expanded T cell receptor clones, increased markers of activation in CD8+ T cells that expressed PD-1, and a decreasing monocytic population in the peripheral blood (p<0.03, two-sided t-test). These findings suggest that the neoadjuvant timing of PD-1 blockade enhances the local and systemic immune response, and may represent a more efficacious approach to the treatment of this uniformly lethal brain tumor.

ATIM-13. ASUNERCEPT PLUS RADIOTHERAPY IN RELAPSED GLIOBLASTOMA. UPDATE ON FIVE YEARS OVERALL SURVIVAL OF STUDY NCT01071837 AND DEVELOPMENT OF A POPULATION-PK - TUMOR GROWTH INHIBITION - SURVIVAL MODEL

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Asunercept (APG101) is an Fc-fusion protein consisting of the extracellular domain of human CD95 (APO-1/Fas) and the Fc domain of IgG1. Asunercept is in clinical development for glioblastoma (GB). Based on PK-data from study NCT01071837 a population pharmacokinetic (PopPK) - tumor growth inhibition (TGI) model was developed and extended to a survival model describing the effect of radiotherapy (RT) or RT + asunercept on the overall survival (OS) of GB-patients. The objective of the model was to identify the best descriptor (i.e. asunercept exposure, tumor size determined by MRT) for survival and to quantify the effect of CpG2 methylation in the CD95 ligand promoter on patient survival.

METHODS: Model development was performed using non-linear mixed effects modeling with NONMEM 7.3 in a stepwise procedure. Firstly, a PopPK–TGI model was developed. Several tumor growth models were tested (e.g. exponential, sequential exponential-linear). Secondly, a survival model was developed and linked to the PopPK-TGI model. RESULTS: For the PopPK-TGI model, data from 84 patients were available contributing to 314 tumor measurements. Glioblastoma growth was best described by an exponential growth model with an average doubling time of 90 days. Asunercept exposure showed a significant inhibitory effect on the tumor growth rate. Tumor size was identified to significantly influence survival. Incorporation of CpG2 CD95L promotor methylation further improved the model: the survival in asunercept-treated patients was prolonged with lower CpG2 methylation status. CONCLUSIONS: A PopPK-TGI-Survival model for asunercept and radiotherapy treated patients was developed. A clear inhibitory effect of asunercept exposure was observable on tumor growth resulting in an increased survival. A recent update on OS of study NCT01071837 revealed that 7% of Asunercept+RT treated 2nd-line GB patients were alive after 5 years compared to 0% in patients treated with RT alone.

ATIM-14. CMV gB/pp65 eVLPs FORMULATED WITH GM-CSF AS A THERAPEUTIC VACCINE AGAINST RECURRENT GLIOBLASTOMA (GBM)

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Cytomegalovirus (CMV) antigens have been reported in over 90% of GBM tumors. $CD4^+$and $CD8^+$ T cells are most frequently directed against the highly immunogenic gB and pp65 antigens. We initiated a phase I/IIa clinical trial for patients with recurrent GBM using gB/pp65 enveloped virus-like particles (eVLPs) formulated with GM-CSF and administered intradermally. In phase I, eligible patients are age 18–70 with KPS at least 70, normal end-organ function, on stable or decreasing corticosteroids of at most 4mg dexamethasone (or equivalent), with recurrent GBM following any standard initial therapy and any number of recurrences. The primary endpoint is safety/tolerability, and secondarily to assess immunogenicity. Additional requirements for phase IIa designed to explore efficacy include unifocal, measurable enhancing tumor 1–3 cm across at first recurrence and no prior immunotherapy. Subjects are vaccinated monthly until tumor progression, with immunomonitoring performed 2 weeks after each vaccination. Up to 3 different vaccine doses will be evaluated, with 6 subjects in each cohort; ten additional subjects will be enrolled once an optimal vaccine dose is identified. To date, 6 patients were accrued 4 men, 2 women, median age 55 (range 39–66) in the first dose cohort. Prior therapies include radiotherapy, temozolomide, and nivolumab. No DLTs were observed. Dose level 1 (0.4µg pp65 content, 200µg GMCSF) is completed and dose Level 2 (2µg pp65 content) is currently accruing. Preliminary analysis of the first 4 subjects demonstrates boosting of CMV-specific antibody titers and T cell responses in two patients, associated with increases (2-3-fold) in plasma levels of CCL3 and proinflammatory INF-g and TNF-a cytokines. These two subjects remain clinically stable without tumor progression after approximately 4 months on study. An expanded immunomonitoring data set will be presented along with associated clinical responses of the subjects.

ATIM-15. A PHASE 1 STUDY OF Ad-RTS-hIL-12 + VELEDIMEX IN ADULTS WITH RECURRENT GLIOBLASTOMA: DOSE DETERMINATION WITH UPDATED OVERALL SURVIVAL

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Ad-RTS-hIL-12 (Ad) is a novel gene therapy expressing IL-12 via the RheoSwitch Therapeutic System® gene switch under control of an oral activator ligand, veledimex (V). We previously reported on an open label Phase I trial describing biological activity of recombinant IL-12 with downstream IFN-γ and activation of the immune system. We provide an update on the intratumoral injections of Ad ($2x10^{11}$virus-particles) + V for patients with recurrent GBM (rGBM) in Group 1 (G1) (craniotomy, n=31) and initial results for Group 2 (G2) (stereotactic administration n=7). In G1, the V 20-mg cohort mOS increased to 12.7 months with mean follow-up of 12.9 months. 20-mg V in G1 showed fewer toxicities and higher V compliance (84%) compared with higher-doses of V (30 and 40-mg) with 75% and 67%, respectively. These

data are encouraging compared to historical data that predict mOS of 5 to 8 months. An additional cohort at V 10-mg (n=6) was well tolerated, but subtherapeutic, with a mOS of 7.6 months (mean follow-up 6.7 months). There was an association between V dose level, blood-brain-barrier penetration, and drug-related adverse events (AEs) with increased TEAEs observed above V 20-mg. Subgroup analyses across all cohorts did not detect statistically significant differences including extent of resection or IDH mutation status. Subjects (20-mg V) who received a cumulative dose of ≤10mg of dexamethasone during the first 15 days of treatment showed improved OS versus >100mg of dexamethasone, suggesting corticosteroid-mediated blunting of the IL-12 dependent immune-mediated therapeutic effect. In the G2 20-mg V cohort, similar cytokine levels and reversible AEs were observed compared to G1; follow up is ongoing and mOS will be presented. Based on these results and the best risk-benefit profile, the 20-mg V dose level was chosen for further investigation. Combination with an immune checkpoint inhibitor in rGBM is underway.

ATIM-16. PHASE 1 STUDY RESULTS OF M7824 (MSB0011359C), A BIFUNCTIONAL FUSION PROTEIN TARGETING TGF- AND PD-L1, AMONG PATIENTS WITH RECURRENT GLIOBLASTOMA (rGBM)

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BACKGROUND: TGF- signaling promotes tumor immunosuppression; TGF- inhibition in the tumor microenvironment may enhance the response to antiPD-L1 treatment. M7824 is an innovative, first-in-class, bifunctional fusion protein composed of a human antiPD-L1 IgG1 monoclonal antibody fused with two extracellular domains of TGF RII to function as a TGF- trap. We report safety and efficacy of M7824 in patients with rGBM. METHODS: In this efficacy expansion cohort of the ongoing, phase 1 trial NCT02517398, patients with rGBM who progressed after chemoradiation received M7824 1200 mg q2w until disease progression, unacceptable toxicity, or trial withdrawal. The primary objective was disease control rate (DCR) per RANO; secondary objectives included safety/tolerability. RESULTS: Among 35 patients, median age was 57 years, 68.6% were male, and 91.4% were at first recurrence. At 15 months minimum follow-up, median treatment duration was 8.1 (range, 2.0–72.1) weeks; four patients remained on treatment at >1 year, and one additional patient decided to stop treatment per protocol at 12 months. Two patients had a partial response, and nine had stable disease (DCR, 31.4% [95% CI, 16.9–49.3]), of which two exhibited early progressive disease and subsequent durable stable disease ongoing for >12 months per investigators assessment. The most common treatment-related adverse events (TRAEs) were gingival bleeding (17.1%), asthenia (14.3%), pruritus, and rash (each 11.4%). Grade 3 TRAEs (6 patients, 17.1%) included diarrhea, eczema, increased liver/pancreatic enzymes, papular rash, papules, one grade 4 asymptomatic increased lipase, and one grade 5 intratumoral hemorrhage (investigator-assessed) coterminous with disease progression. CONCLUSIONS: M7824 demonstrated a manageable safety profile and encouraging efficacy in rGBM, including two durable partial responses and a DCR of 31.4%. Further investigation of M7824 in GBM is warranted; future development aims to define molecular characteristics of responders.

ATIM-17. PEMBROLIZUMAB BLOCKS PD-1 ON CAR T CELLS ADMINISTERED INTRAVENTRICULARLY TO GLIOBLASTOMA PATIENTS

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BACKGROUND: Checkpoint inhibitors have shown efficacy in other solid tumors and are being studied in glioblastoma. For patients treated with anti-PD-1 monoclonal antibodies, such as pembrolizumab, CSF concentrations and blockade of PD-1 on T cells present in the CSF have not been reported. Such information would provide valuable context for applying checkpoint inhibitors for the treatment of CNS malignancies. METHODS: CSF and blood samples were obtained from 6 glioblastoma CAR T cell study patients who received intraventricularly administered CAR T cells. These patients were also treated with pembrolizumab 200 mg intravenously every 3 weeks. Pembrolizumab levels in CSF and blood were measured using an ELISA assay. FACS analysis for PD-1 blockade was performed on endogenous and CAR T cells detected in CSF samples, and the results were compared to cells in CSF without pembrolizumab treatment. RESULTS: Data analyzed from 3 patients samples so far show that average pembrolizumab levels in the CSF and serum were 330 ± 114 ng/mL and 64 ± 8 µg/mL, respectively. The average CSF/serum ratio was 0.5 ± 0.1%. In 1 patient from whom multiple CSF/blood samples were obtained over 3 months, steady-state CSF levels ranged from 155–394 ng/mL throughout each 21 day cycle of pembrolizumab. PD-1 was completely blocked on both endogenous T cells as well as CAR T cells that were delivered directly into the CSF. CONCLUSIONS: To our knowledge, this is the first report of pembrolizumab concentrations in the CSF after intravenous administration. CSF levels were 0.5% of serum concentrations and remained stable throughout a 21 day cycle of pembrolizumab. PD-1 was found to be blocked on endogenous T cells in the CSF. Furthermore, even though they were relatively low, pembrolizumab levels in the CSF were sufficient to block PD-1 on intraventricularly administered CAR T cells.

ATIM-18. A PHASE I TRIAL OF VACCINATION WITH AUTOLOGOUS DENDRITIC CELLS PULSED WITH LYSATE DERIVED FROM AN ALLOGENEIC GLIOBLASTOMA STEMLIKE CELL LINE FOR PATIENTS WITH NEWLY DIAGNOSED OR RECURRENT GLIOBLASTOMA

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INTRODUCTION: We conducted a single-institution phase 1 trial of a dendritic cell immunotherapy targeting glioblastoma stem-like cells for patients with either newly diagnosed or recurrent glioblastoma. METHODS: Following gross total or near-gross total resection, patients who consented to participate in this trial underwent leukapheresis for isolation of peripheral blood mononuclear cells, which were then differentiated into dendritic cells in culture, pulsed with lysate derived from an allogeneic glioblastoma stem-like cell line, and administered by intradermal injection weekly x 4 weeks, then every other month until disease progression or vaccine depletion. Patients with newly diagnosed glioblastoma were treated with standard-of-care chemoradiation, with vaccine injections beginning 1 week following the completion of radiation therapy. Patients with recurrent disease received no other disease-directed therapy while on trial. RESULTS: From December 2013 to February 2018, 38 patients enrolled in this trial -- 12 patients with newly diagnosed glioblastoma and 26 with recurrent disease. Median age 57 (range 19–77), median KPS 80 (range 70–100), 66% male. Survival functions were estimated using the Kaplan-Meier method. For newly diagnosed patients, median Time-to-Progression (TTP) was 8.86 mo, and median Overall Survival (OS) was 21.1 mo. For patients with recurrent glioblastoma, median TTP was 3.14 mo and median OS was 12.0 mo. Treatment was well-tolerated with no related grade 3/4 toxicities. As of this analysis, 7 patients are still alive, and 2 patients in the newly diagnosed glioblastoma cohort are still progression-free. Immune response studies and tumor antigen profiling are ongoing. CONCLUSION: Consistent with other previously completed dendritic cell immunotherapy trials, this phase 1 trial demonstrates improved TPP and OS for patients with either newly diagnosed or recurrent glioblastoma compared to historical controls. Ongoing efforts include characterizing and expanding the subset of patients who most benefit from immunotherapy.

ATIM-19. RESULTS OF THE GLOBE STUDY: A PHASE 3, RANDOMIZED, CONTROLLED, DOUBLE-ARM, OPEN-LABEL, MULTI-CENTER STUDY OF VB-111 COMBINED WITH BEVACIZUMAB VS. BEVACIZUMAB MONOTHERAPY IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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BACKGROUND: Ofranergene obadenovec (VB-111) is a viral cancer-therapy with a dual mechanism: vascular disruption and induction of a tumor directed immune response. In a phase 2 trial, recurrent GBM (rGBM) patients treated with VB-111, followed at progression with VB-111 in combination with bevacizumab (BEV), had durable tumor growth attenuation,

associated with prolonged overall survival (OS) of 15 months. METHODS: A phase 3 multisite international randomized open-labeled controlled trial. Patients with rGBM were randomized 1:1 to receive VB-111 at 10e13VPs q8W in combination with BEV 10mg/Kg q2W vs. BEV 10mg/Kg q2W. Primary endpoint was OS. RESULTS: 256 patients (128 per arm) were enrolled in 57 sites. The mean age was 55, 67% were male. 74% were in 1st progression, KPS < 80 found in 23% of patients. In the combination arm vs the BEV arm: baseline tumor volume > = 15cm3 in 49% vs 41%; Grade 3–4 adverse events reported among 61 % (mostly CNS and febrile) vs 34%, and pyrexia in 39% vs 4% of patients; ORR was 27.3% vs 21.9% and median duration of response was 3.7 vs 2.2 months. Median OS was 6.8 vs 7.9 months in the combination vs BEV arms, HR 1.2 [95% CI 0.910–1.59, p=NS]. In the subgroup of patients with baseline tumors< 15 cm3, OS was 9.2 vs 8.3 months [p=NS], and 12 month OS was 38.9% vs 26.9% [p=NS]. Among patients in the combination arm, OS was 7.9 vs 5.5 in patients with and without a febrile reaction. CONCLUSIONS: In this trial, VB-111 in combination with BEV failed to increase OS in patients with rGBM. Lack of VB-111 priming, as done in the phase II trial may explain the differences with the favorable outcomes in the latter. Patients with large progressive tumors may precluded sufficient drug exposure. Additional exploratory analyses are ongoing.

ATIM-20. GAPVAC-101 TRIAL OF A HIGHLY PERSONALIZED PEPTIDE VACCINATION FOR PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: There is a need for treatment personalization as every cancer is molecularly unique. In addition glioblastoma (GB) are immunologically regarded as resistant, cold tumor with few targetable antigens available from mutations, thus demanding new personalized immunotherapies. So far outside Neuro-Oncology, T cells orchestrate impressive anti-tumor effects with checkpoint inhibitors, but also vaccines. METHODS: The GAPVAC consortium established an immunotherapy, for which personalized selection of 2 peptide-based actively personalized vaccines (APVAC) per patient for treatment of newly diagnosed GB was based not only on whole-exome sequencing but also on human leukocyte antigen (HLA)-ligandome analyses providing insight into the actual presentation of relevant epitopes in the tumor. GAPVAC-101 (NCT02149225) enrolled 16 patients in a European phase I feasibility, safety and immunogenicity trial integrated into standard of care. For APVAC1, up to 7 peptides were selected from a trial specific warehouse based on individual biomarker data. Vaccination (i.d.) with GM-CSF and poly-ICLC in 15 patients started with the 1st adjuvant cycle of temozolomide (TMZ). For APVAC2, analyses revealed a median of 36 somatic, non-synonymous mutations in the patients tumors. From the 4th TMZ cycle, 11 patients received APVAC2 with usually 2 de novo antigens per patient selected according to mutation, actual or putative HLA presentation and immunogenicity. Overall 20 APVAC2 antigens incl. 14 mutated were vaccinated. RESULTS: Adverse events were largely reversible injection site reactions and two anaphylactic reactions and one increase in cerebral edema. Short, non-mutated APVAC1 antigens induced sustained CD8 responses with memory phenotype. Mutated APVAC2 antigens induced predominantly CD4 responses of favorable TH1 type. Median PFS and OS were 14.2 and 29 months from diagnosis, respectively, in patients that received 1 APVAC vaccination (N=15). CONCLUSION: Overall, GAPVAC displayed expected safety profiles and high biological activity indicating further development.

ATIM-21. UPDATED RESULTS OF A PHASE I TRIAL OF ANTI-LAG-3 OR ANTI-CD137 ALONE AND IN COMBINATION WITH ANTI-PD-1 IN PATIENTS WITH RECURRENT GBM

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BACKGROUND: Others and we have shown additional checkpoint molecules are expressed in GBM and preclinical studies have shown that combining anti-Lag-3 and anti-CD137 with anti-PD-1 can induce effective antitumor immune responses. METHODS: The Adult Brain Tumor Consortium (ABTC) 1501 trial is a phase I, open label, multicenter, multi-arm dose-finding/safety study of anti-LAG-3 (BMS-986016) or anti-CD137 (BMS-663513) alone and in combination with anti-PD-1 in patients with first time recurrent GBM. The primary objective was to define MTD for the mono and combination treatments with a secondary objective of OS. Using a sequential allocation, we started with doses of 80mg for anti-LAG-3 and 8mg flat for anti-CD137. Anti-PD-1 was given at a flat dose of 240 mg in the combination treatment arms. Using a 3 + 3 design our target DLT rate was < 33%. RESULTS: To date 30 patients were enrolled into the trial with median age at 56, median KPS of 90. Median treatment cycle was 3 and 43% tumors were MGMT methylated. Recruitment of the monotherapy Anti-LAG-3 and anti-CD137 arms were completed. We observed no DLT at the highest dose for Anti-LAG-3 at 800mg and only one DLT (a grade 3 elevated serum ALT at end of cycle 2) with anti-CD137 at the top dose of anti-CD137 at 8mg flat. In addition, three out of 12 patients developed elevated ALT at end of cycle 2 that was considered possibly related to anti-CD137. Another two patients had grade 1 elevated ALTs. Six patients are currently enrolled into a combination cohort of Anti-LAG-3 at160mg +anti-PD-1with no observed DLT and the combination arm of anti-CD137 with anti-PD-1 is open to accrual. CONCLUSIONS: The safe monotherapy dose is 800mg for anti-LAG-3 and 8mg flat for anti-CD137. Both Anti-LAG-3 and anti-CD137 in combination with antiPD-1 cohorts and an intratumoral surgical anti-CD137 cohort are open for accrual.

ATIM-22. PROGNOSTIC VALUE OF PTEN LOSS IN NEWLY DIAGNOSED GBM PATIENTS TREATED WITH AUTOLOGOUS HEAT SHOCK PROTEIN VACCINE

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INTRODUCTION: The safety and efficacy of a heat shock protein peptide complex- 96 vaccine (HSPPC-96, Prophage) has been previously studied in phase II single-arm trials for the treatment of newly diagnosed and recurrent glioblastoma (GBM). These studies demonstrated modest improvements in survival compared with historical standards. PTEN loss has been recently associated with immunoresistance in GBM patients, mediated in part by B7-H1. PTEN status has not shown clear prognostic value in GBM patients treated with standard of care therapies. The aim of this study is to evaluate the prognostic significance of PTEN status in newly diagnosed GBM patients treated with autologous HSP vaccine and standard chemoradiation. METHODS: Our institutional cohort of patients enrolled in a single arm, phase II study of adult GBM patients treated with autologous HSP vaccine and standard chemoradiation (n=27) was analyzed. Differences in overall survival (OS) by PTEN status were evaluated via Kaplan-Meier curves and Log-rank test. RESULTS: Median overall survival (n=27) was 26 months. 23 patients had PTEN status available. PTEN loss was found in 16 patients (69.6%) whereas retained PTEN was present in 7 patients (30.4%). Median OS was 59 months (95% CI, 0–120 months) in patients with retained PTEN and 23 months (95% CI, 15–30 months) in patients with PTEN loss. The difference in OS was statistically significant (p=0.037). CONCLUSION: Retained PTEN expression was associated with extended survival in GBM patients treated with HSP vaccine. This finding suggests that PTEN loss may be associated with resistance to vaccine treatment and emphasizes the need for subgroup analysis in further immunotherapy studies.

ATIM-23. ANTI-CD27 AGONIST ANTIBODY VARLILUMAB IN COMBINATION WITH NIVOLUMAB FOR RECURRENT GLIOBLASTOMA (rGBM): PHASE 2 CLINICAL TRIAL RESULTS

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CD27 is a key immunostimulatory molecule that enhances T cell survival, activation and effector function, as well as proliferation and cytotoxic activity of NK cells. Preclinical studies demonstrate synergistic activity of PD-(L)1 blockade and varlilumab, an anti-CD27 agonist monoclonal antibody. In an open-label Phase 2 study (NCT02335918), patients with bevacizumab-naïve, rGBM following first line chemoradiation received varlilumab (3 mg/kg, up to 16 doses) and nivolumab (240 mg) Q2W. Objectives were to determine preliminary antitumor activity based on 12-month survival rate (OS12; primary), objective response rate (ORR; iRANO), progression-free survival (PFS), as well as tolerability. 22 patients were enrolled: 23% methylated MGMT (mMGMT), 68% unmethylated MGMT (uMGMT), 9% unknown; median age 58 years; 68% male; 36% ECOG performance status 0, 64% ECOG 1; 4/18 (22%) PD-L1+ tumor. Safety profile was consistent with that of each agent alone and generally grade 1–2, without dose-limiting toxicity or drug-related deaths. The most common toxicities were lymphopenia, pruritus, headache, and rash. Two patients experienced treatment-related serious events (grade 2 gait disturbance, headache and personality changes; and grade 4 thrombocytopenia). OS12 was 38.5% (95% CI; 18.6, 58.2) overall and 43.6% (95% CI; 18.2–66.7) for the uMGMT subgroup. Median OS (months) was 9.7 (95% CI; 6.7–14.8) overall and 11.3 (95% CI; 5.3, -) for the uMGMT subgroup. Eight patients (6 uMGMT, 1 mMGMT, 1 unknown) survived 12 months (range: 13.7 - 23+). Two patients (9%; both uMGMT) experienced Partial Responses (63% and 92% shrinkage) continuing at 19 and 16 months. Nine patients experienced stable disease, including 5 for 6 months (range: 7.3 - 20.3). Varlilumab with nivolumab was generally well tolerated in patients with rGBM and achieved durable therapeutic benefit in a subset of patients, although overall ORR and OS12 were similar to historical nivolumab monotherapy data. Outcome among uMGMT rGBM patients may be encouraging.

ATIM-24. INTERIM RESULTS OF A PHASE II MULTICENTER STUDY OF THE CONDITIONALLY REPLICATIVE ONCOLYTIC ADENOVIRUS DNX-2401 WITH PEMBROLIZUMAB (KEYTRUDA) FOR RECURRENT GLIOBLASTOMA; CAPTIVE STUDY (KEYNOTE-192)

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BACKGROUND: DNX-2401 (tasadenoturev) is a replication-competent, tumor-selective, oncolytic adenovirus. A dose-escalating, Phase II study of a single intratumoral injection of DNX-2401 with pembrolizumab to determine optimal dose, safety and efficacy is ongoing and enrolling up to 48 patients with recurrent glioblastoma. Key inclusion criteria include patients with a single lesion at first or second recurrence for which resection is not possible or planned. METHODS: In a dose-escalation design, a single intratumoral dose of DNX-2401 (5e8vp, 5e9vp, 5e10vp) is administered via cannula, followed 7 days later by 200 mg pembrolizumab Q3wk for up to 24 months or until confirmed progression, intolerable toxicity, or study withdrawal. Tumor response is assessed every 4 weeks for 6 months and every 8 weeks thereafter. RESULTS: As of 01May2018, 23 patients have been treated, with 17 at the optimal dose of 5e10vp DNX-2401. The median age and KPS at entry was 52 years (26–65) and 90 (80–100), respectively. The most frequent related grade 3–4 AEs across cohorts are headache (30%), fatigue (9%), and increased GGT (9%) consistent with disease or immune activation. Transient grade 1–3 lymphopenia has also been observed. Several cases of vasogenic edema have been managed with steroid tapers or low-dose bevacizumab. No patient has died or discontinued due to study treatment. The median treatment duration is 5.1 months for evaluable patients treated with DNX-2401 and pembrolizumab (N=15). Preliminary efficacy includes two partial responses and 100% 9-month survival for the first 7 patients treated. CONCLUSIONS: DNX-2401 followed by pembrolizumab is well tolerated, and data emerging for disease control and survival are encouraging. Updated results will be presented.

ATIM-25. NEOADJUVANT PD-1 ANTIBODY BLOCKADE IS ASSOCIATED WITH FOCAL UPREGULATION OF PD-L1 AND CD8 T CELL INFILTRATE IN RECURRENT GLIOBLASTOMA

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BACKGROUND: The use of anti-PD-1 therapy, pembrolizumab, has shown minimal therapeutic effect as an adjuvant treatment in glioblastoma, but its efficacy as a neoadjuvant in recurrent glioblastoma setting has yet to be established. METHODS: The Ivy Foundation Early Phase Clinical Trials' Consortium conducted a randomized, multi-institution Phase I clinical trial to evaluate the immune response and survival following neoadjuvant and adjuvant therapy with pembrolizumab in thirty patients with recurrent, surgically resectable glioblastoma. Formalin-fixed paraffin embedded tissue was stained using multiplex immunohistochemistry to spatially visualize and quantify the following markers: CD8, PD-1, CD45, GFAP, and PD-L1. RESULTS: The density of tumor infiltrating CD8 T-cells was not statistically different between groups, but distinctly variable in the neoadjuvant treatment group. PD-1 expression across both treatment groups co-localized with CD8 T-cells, though 9 neoadjuvant and only 3 adjuvant samples had appreciable PD-1 detected. Samples with the highest percentage of PD-L1 expression and double positive CD8/PD-1 cell population were present in both groups. Notable CD45/PD-L1 populations were found in 3 neoadjuvant treated samples, with only 1 in the adjuvant group. Samples were classified as having either constitutive, focal, or negative PD-L1 expression pattern with varying degrees of CD8 infiltrate. 7 neoadjuvant patients and 3 adjuvant patients exhibited a focal phenotype with a high CD8 infiltrate. Neoadjuvant samples exhibiting focal PD-L1 expression also had a higher median survival compared to the corresponding adjuvant group ($p=0.035$, Mantel-Cox log rank). CONCLUSION: Classification of tumors based on PD-L1, CD8, and PD-1 IHC may be predictive of outcome and therapeutic effect. We identified a subset of patients with focal expression of PD-L1 with increased survival. This suggests that focally expressed PD-L1, perhaps induced on tumor and immune infiltrate by the presence of an anti-tumor CD8 response, is a biomarker that is predictive of a positive response to anti-PD-1 checkpoint blockade.

ATIM-26. IMMUNOLOGIC TRENDS ASSOCIATED WITH PATIENT OUTCOMES IN A PHASE 1 CLINICAL TRIAL OF TOCA 511 AND TOCA FC IN RECURRENT HIGH GRADE GLIOMA

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Toca 511 (vocimagene amiretrorepvec) is a cancer selective, retroviral replicating vector encoding a codon optimized, heat stabilized cytosine deaminase that converts Toca FC (extended-release 5- fluorocytosine, 5-FC) into the anticancer agent 5-fluorouracil. Preclinical evidence demonstrates that the Toca 511 & Toca FC regimen kills cancer cells and immunosuppressive myeloid cells in the tumor microenvironment, leading to durable

antitumor immune responses that can be adoptively transferred to untreated animals. In an ascending dose trial (NCT01470794) in patients with recurrent high grade glioma (rHGG), Toca 511 was injected into the resection cavity walls at the time of resection, and then multiple courses of oral Toca FC were administered. Multiyear durable and complete responses by independent radiology review have been reported. Human immune monitoring results support an immunologic mechanism of action and identify potential biomarkers related to patient outcomes. Measurements included the quantification of peripheral blood and tumor infiltrating leukocyte subsets by flow cytometry, immunohistochemistry, and deconvolution of DNA and RNA sequencing data. In addition, systemic cytokine levels were assessed in peripheral blood serum by multiplex digital ELISA. Univariate comparisons and multivariate models revealed immunologic trends associated with patient outcomes. Pre-treatment tumor infiltrating cell subsets, quantified via deconvolution of RNA sequencing data, were associated with both objective responses and survival. Subsequent exploratory models applied to selected patient data indicate that a combined biomarker using mRNA signatures from multiple leukocyte subsets may predict patient outcomes with high sensitivity and selectivity. In addition, post-treatment serum cytokine time-course results suggest that differences and temporal modulations are associated with both objective response and survival. These results support an immune-related mechanism of action for the Toca 511 & Toca FC regimen. Potentially predictive and/or prognostic biomarkers of patient outcomes will be evaluated in the ongoing randomized Phase 3 Toca 5 trial in patients with rHGG (NCT02414165).

ATIM-27. INTRATUMORAL ADMINISTRATION OF AN ONCOLYTIC POLIO/RHINOVIRUS RECOMBINANT (PVSRIPO) IN MALIGNANT GLIOMA PATIENTS: ASSESSMENT OF MUTATIONAL RESPONSE CORRELATES

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BACKGROUND: The live attenuated oral poliovirus vaccine was modified to contain a heterologous internal ribosomal entry site stemming from human rhinovirus type 2, creating PVSRIPO. PVSRIPO recognizes CD155, an oncofetal cell adhesion molecule and tumor antigen widely expressed ectopically in malignancy. We report results of the dose finding trial evaluating PVSRIPO delivered intratumorally by convection-enhanced delivery (CED). METHODS: Eligible patients were adults with recurrent supratentorial WHO grade IV MG; solitary tumor 1–5.5cm in diameter; 4 weeks after chemotherapy, bevacizumab or study drug; adequate organ function; KPS70%; and positive anti-polio titer. RESULTS: A total of 61 pts were treated on study. Only one DLT was observed, a grade 4 intracranial hemorrhage at the time of catheter removal on DL5. Study related adverse events consisted of localized peritumoral inflammation, triggering neurologic symptoms in relation to the location of the infused tumor. Of the 26 patients treated more than 36 months ago, six are alive at 73.6+, 72.5+, 60.6+, 44.0+, 39.3+, and 36.9+ months. Deep sequencing of biopsy material obtained prior to PVSRIPO infusion in 31 samples, confirmed that a very low mutational load is associated with longer survival (p=0.017). Additionally, no patients whose tumors had >0.5 non-synonymous mutations per Mb survived beyond 18 months. CONCLUSION: Infusion of PVSRIPO via CED is safe and encouraging efficacy results were observed in adults along with mutational correlates of response.

ATIM-28. PHASE 2 STUDY OF ERC1671 PLUS BEVACIZUMAB VS BEVACIZUMAB PLUS PLACEBO IN RECURRENT GBM INTERIM RESULTS AND CORRELATIONS WITH CD4+ T LYMPHOCYTE COUNTS

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BACKGROUND: ERC1671 is an allogeneic/autologous therapeutic glioblastoma (GBM) vaccine composed of whole, inactivated tumor cells mixed with tumor cell lysates derived from the patient and three GBM donors. Previously published compassionate use data showed an overall survival (OS) of 43 weeks (10 months) in patients with a good performance status. METHODS: In this double-blinded, randomized, phase 2 study bevacizumab-naïve patients with recurrent GBM were randomized after surgery to receive either ERC1671 in combination with GM-CSF and cyclophosphamide plus bevacizumab, or placebo plus bevacizumab. The trial is registered with ClinicalTrials.gov (NCT01903330). Interim RESULTS: Nine patients, with a KPS 70, were randomized and treated. At the time of further progression, these patients were unblinded, as stipulated by the protocol, which revealed that four had received vaccine, four had received placebo, and one was non-evaluable. Median OS of patients treated with ERC1671 plus bevacizumab was 12 months, with one patient surviving >2 years. In the group treated with placebo plus bevacizumab, median OS was shorter at 7.5 months, with all patients having succumbed within 1 year. Toxicity analysis showed an equal distribution of adverse events (AE) between the vaccine and placebo groups, with no grade 4 or 5 toxicities. The maximal CD4+ T lymphocyte count in the peripheral blood correlated with OS in the ERC1671 but not in the placebo group. CONCLUSIONS: The addition of ERC1671/GM-CSF/cyclophosphamide to bevacizumab resulted in a potential survival benefit with minimal additional toxicity. The maximal CD4+ T lymphocyte count in the peripheral blood correlated with OS. The study is ongoing with the addition of two other sites.

ATIM-29. NRG BN002: SAFETY DATA FROM A PHASE I STUDY OF IPILIMUMAB (IPI), NIVOLUMAB (NIVO), AND THE COMBINATION FOR NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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INTRODUCTION: Immune checkpoint inhibitors (ICIs) have demonstrated efficacy in several solid tumors including brain metastases, but single agent ICIs have failed to improve outcome in recurrent (GBM). This study evaluated the safety of anti-CTLA-4 (IPI) and anti-PD-1 (NIVO) ICIs alone or in combination in newly diagnosed GBM during adjuvant temozolomide (TMZ) treatment. METHODS: This phase I study evaluated IPI (3mg/kg), NIVO (3mg/kg), and the combination (1 mg/kg & 3 mg/kg respectively) followed by an expansion cohort for the combined treatment of adults with unifocal, supratentorial newly diagnosed GBM after gross or near total resection. ICIs were given with adjuvant TMZ. The primary endpoint was the dose limiting toxicity (DLT) within 8 weeks of starting ICIs. A standard up-and-down design was used with 6 evaluable patients enrolled at a given dose level; safety defined as 1 or fewer patients with DLTs. RESULTS: Thirty-two patients (31 analyzable; 1 not treated) were enrolled, 6 to each arm and 14 to the expansion cohort. Median age: 54 years (range: 23–74), 68% male and 84% white. Overall, treatment was well tolerated with a 16% rate of Grade 4 events; without increased toxicity of combination ICIs; there were no Grade 5 events. One DLT was seen in each single-agent arm; none in the combination arm. Median follow-up time was 8.4 months (range: 0.5–23.6), 10 had progressed (32%) and 8 had died (26%), 7 due to disease progression and 1 due to pulmonary embolism. CONCLUSIONS: IPI and NIVO are safe and tolerable with similar toxicity profiles noted with other cancers when given with adjuvant TMZ for newly diagnosed GBM. Combination IPI+NIVO is not more toxic than single agents. These results provide necessary safety data for a subsequent efficacy trial to test the combination of ICIs in newly diagnosed GBM. Funding: U10CA180868 and U10CA180822 (NCI).

ATIM-30. HOW TO MONITOR IMMUNOGENIC CELL DEATH IN PATIENTS WITH GLIOBLASTOMA

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Immunotherapy approaches become prominent in the treatment of patients with GBM. One pathway is the induction of immunogenic cell death of tumor cells (ICD). Instant monitoring of the effects of ICD might help the physician to continuously adapt these therapies. For this, blood liquid biopsy like biological biopsy might help. The presence and evolution of tumor-derived TKTL1 and Apo10 in circulating monocytes, both components of the PanTum tests using the EDIM platform, were studied in a series of 57 patients with glioma treated with 2 cycles of multimodal immunotherapy consisting of modulated electrohyperthermia (mEHT), injections of Newcastle Disease Virus (NDV), autologous mature dendritic cell vaccinations loaded with autologous tumor antigens, and immunomodulatory strategies depending on the functioning immune system. Median age was 44 years (ranging 1–73). Patients had high grade glioma (n=48), grade II glioma (n=3), DIPG (n=4), ependymoma (n=1) and metastasized paraganglioma (n=1). A significant increase of TKTL1 and Apo10 was measured after 5 days of mEHT/NDV-based ICD treatment. Three weeks later, the markers were dropped but rose again significantly upon ICD treatment. Increase was observed for all disease entities. Values were relatively lower in lower grade tumors. The effect of ICD can be monitored through TKTL1 and Apo10 measurements. Whether the dynamics of these markers upon treatment predict efficacy of multimodal immunotherapy is focus of current research.

ATIM-31. PHASE I STUDY OF TUMOR TREATMENT FIELDS AND A PERSONALIZED MUTATION-DERIVED TUMOR VACCINE IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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The tumor microenvironment of a glioblastoma is highly immunosuppressive. Overcoming this immunosuppressive activity seems imperative to a successful treatment. Like other cancers, glioblastoma is capable of generating tumor-specific peptides that can be recognized by the immune system, providing a specific target for antitumor therapy. Glioma vaccines including tumor lysate pulsed dendritic cell-based immunotherapy have proven safe and potentially of clinical value, suggesting that dead cells can confer tumor associated antigens for processing and presentation. Use of TTFields in newly diagnosed glioblastoma increases progression-free survival and overall survival. Among several mechanisms of action, TTFields induces an immunogenic cell death. Therefore, we combined this modality treatment with the standard of care and a personalized vaccine for newly diagnosed glioblastoma with the expectation of improved outcome for the patients. RNA and DNA are extracted from tumor specimens and blood and whole exome sequence and mRNASeq is performed. HLA typing is obtained for each patient. Mutation-derived tumor antigens are identified using computational predictions and ranked according to both predicted HLA binding affinity and expression. A maximum of 10 peptides is selected for each patient vaccine. The vaccine is administered subcutaneously using Poly-ICLC as adjuvant during Temozolomide treatment after radiotherapy with concurrent Temozolomide. We are reporting on the initial safety data for patients who received the mutation-derived tumor antigen vaccine without the use of the TTFields. We have encountered not only novel mutated tumor antigens but also well known driver mutations that are predicted to be immunogenic despite a low mutation load in the GBM patients. The trial is feasible and the vaccine seems well tolerated without unexpected adverse events.

ATIM-32. PERSONALIZED NEOANTIGEN-TARGETING VACCINE GENERATES ROBUST SYSTEMIC AND INTRATUMORAL T CELL RESPONSES IN GLIOBLASTOMA (GBM) PATIENTS

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BACKGROUND: The impact of individualized neoepitope vaccination targeting neoantigens arising from tumor-specific mutations for GBM, a low mutation burden tumor with an immunologically cold tumor microenvironment, as well as that of concurrently administered dexamethasone (dex), are unknown. METHODS: Individualized vaccination of up to 20 synthetic, long neoepitope peptides with high predicted HLA binding affinity admixed with poly-ICLC, were administered subcutaneously using a prime-boost schedule after RT to newly diagnosed, MGMT unmethylated, at least partially resected, GBM patients without progression after radiation (RT) in our phase 1b study. RESULTS: 9 of 10 screened patients had sufficient (10) identified neoepitope peptides. 8 patients without progression after RT received vaccine consisting of a median of 12 peptides (range, 7–20) beginning a median of 19.9 wks (range 17.1–24.7) after surgery. Adverse events were limited to infrequent grade 1/2 local reactions and fatigue. Median PFS and OS were 7.5 mths (90% CI: 6.2, 9.7) and 16.8 mths (90% CI: 9.6, 21.3). Evaluation of neoepitope-specific immune responses and tumor immune infiltrate analyses were performed on five patients with pre- and post-vaccination samples. Three patients on dex for post-RT edema during vaccine had no immune responses and no change in tumor infiltrating effector cells. In contrast 2 patients not on dex had robust, de novo immune responses against multiple predicted personal neoantigens including polyfunctional neoantigen-specific CD4+ and CD8+ T cell responses that were enriched for memory and activated phenotypes as well as increased numbers of tumor-infiltrating CD4+ and CD8+ T cells. T cell receptor analysis from one patient identified identical clonotypes isolated from post-vaccination tumor tissue and peripheral blood including two clonotypes specific for ARHGAP35, a neoantigen targeted by vaccination. CONCLUSIONS: Individualized, multi-neoepitope vaccines are feasible, safe and capable of generating systemic and intra-tumoral immune responses in GBM patients that appear to be abrogated by dex.

ATIM-33. NOA-16: A FIRST-IN-MAN MULTICENTER PHASE I CLINICAL TRIAL OF THE GERMAN NEUROONCOLOGY WORKING GROUP EVALUATING A MUTATION-SPECIFIC PEPTIDE VACCINE TARGETING IDH1R132H IN PATIENTS WITH NEWLY DIAGNOSED MALIGNANT ASTROCYTOMAS

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In preclinical studies we have defined IDH1R132H as a clonal neoantigen presented on MHC class II. A peptide vaccine encompassing IDH1R132H induces tumor-specific T helper cell responses effective in controlling syngeneic IDH1R132H-mutant tumors in humanized mouse models. NOA-16 (NCT02454634) is a first-in-man, multicenter, phase I trial testing the safety and immunogenicity of an IDH1R132H peptide in incomplete Freunds adjuvant in patients with newly diagnosed, IDH1R132H mutant WHO °III and °IV astrocytomas. Between September 2015 and October 2016, 32 patients were enrolled in seven German sites. 23 patients (71.9%) received radiochemotherapy with temozolomide, six patients (18.8%) received radiotherapy alone and three patients (9.4%) received temozolomide alone. 249 vaccines were administered, 29 (90.6%) of the patients of the safety set (N=32) and 27 (90.0%) patients of the immunogenicity set (N=30) received all eight vaccines. No regime-limiting toxicity was observed. The majority of the patients (N=29, 90.6%) experienced treatment related adverse events (trAE), 1 (3.1%) of them had treatment related SAE. None of the reported AEs were severe. 28/30 (93.3%) patients, who were evaluable for immuno-

genicity, displayed mutation-specific T cellular (24/30 (80%)) or humoral (26/30 patients (87%)) immune responses not detectable before vaccination. Until end of study no deaths were observed. 4/32 (12.5 %) patients had PD according to RANO criteria, all other patients (N=28, 87.5%) had SD. 12/32 (37.5%) patients displayed pseudoprogressions after the initiation of the vaccine. Single-cell T cell receptor (TCR) sequencing allowed for the identification of IDH1R132H-specific TCRs. In conclusion, NOA-16 met its primary endpoints by demonstrating safety and immunogenicity of a mutation-specific IDH1R132H peptide vaccine given with standard of care in patients with newly diagnosed IDH1R132H mutant malignant astrocytoma.

ATIM-34. TARGETING THE CD200 CHECKPOINT FOR THE FIGHT AGAINST CENTRAL NERVOUS SYSTEM TUMORS

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Numerous ongoing clinical trials targeting immune checkpoints have failed to enhance survival in patients with CNS tumors. We are targeting a unique checkpoint, CD200, which controls the immune system through paired inhibitory and activation receptors. The CD200 checkpoint interferes with tumor-immune interactions through multiple mechanisms: i) CD200 is secreted from tumors inducing an immunosuppressive environment, ii) CD200 is upregulated in tumor-associated vascular endothelial cells, creating an immunological barricade around the tumor microenvironment. We are targeting the activation receptor with a peptide ligand (CD200AR-L) activates antigen-presenting cells, enhancing dendritic cell maturation, cytokine production and antigen specific T cell activation. Treatment with CD200AR-L significantly extends survival in two murine glioma models. Directing the immune system to fight the tumor, requires introduction of an antigen such as autologous or allogeneic tumor lysate for non-immunogenic tumors such as CNS tumors. In an ongoing pilot study treating companion dogs with high-grade glioma, patients receiving a canine specific CD200 peptide inhibitor in combination with the autologous tumor lysate vaccine increased median survival to 330 days, compared to 194 with lysate alone. Currently, 41% of the dogs are alive; the longest living dog is now 810 days post-surgery. 28% of dogs died of non-tumor related deaths. In contrast, 100% of the dogs in the tumor lysate-only group died of tumor recurrence. Furthermore, serum chemistry profiles and physical examinations showed that the peptide did not induce any systemic toxicity. Importantly, we have developed human CD200 peptide ligands that enhance cytokine secretion, dendritic cell maturation, and antigen-specific immune response. This innovative research may provide a significant breakthrough for the field of cancer immunotherapy. We have also demonstrated that the use of a CD200AR-L in a murine breast carcinoma model resulted in a significant survival benefit. In this light, CD200AR-L may be a powerful immunotherapy platform for other solid tumors.

ATIM-35. VXM01 PHASE I STUDY IN PATIENTS WITH PROGRESSIVE GLIOBLASTOMA – FINAL RESULTS

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BACKGROUND. VXM01 consists of the attenuated *Salmonella* Typhi Ty21a delivering a plasmid encoding vascular endothelial growth factor receptor 2 (VEGFR2) into the Peyer's patches *via* the oral route of administration. The vaccine elicits a systemic T-cell response targeting VEGFR2. This trial examined safety and tolerability, clinical and immunogenic responses to VXM01 after at least four vaccinations at 10^6 or 10^7 colony-forming units in patients with progressive glioblastoma who have failed at least radiochemotherapy with temozolomide. METHODS. Patients with progressive operable glioblastoma were subjected to VXM01 in one oral administration each on day 1, 3, 5, and 7, and 4-weekly single doses during the tumor follow-up period after surgery. Follow-up was done by safety laboratories and physical examinations, MRI, T-cell immunomonitoring in the peripheral blood, and brain tumor immunohistochemistry. RESULTS. Fourteen patients have been treated with VXM01, three out of them with additional nivolumab. Surgery has been performed in eight patients. Under VXM01 treatment 129 adverse events, mostly unrelated to VXM01, were observed after a median of 7.5 doses per patient. IFN-γ ELISpot analysis showed a detectable VEGFR2–specific T–cell response in 7 out 12 patients measured. In the observation period up to 2 years, seven patients are alive and survived for more than 12 months. Survival correlated with a higher CD8/Treg ratio in progressive and primary tumor, which further increased after VXM01 treatment. In patients with prolonged survival a decrease in intratumoral PD-L1 was measured. In one patient, a strong partial response was observed under VXM01 monotherapy, and a complete response after addition of nivolumab. CONCLUSION: VXM01 was safe, produces detectable specific peripheral immune responses and increased T-cell infiltration in post-vaccine tumor tissue, with a favorable course of disease in five patients. A combination study of VXM01 and anti-PD-L1 avelumab in 30 patients with relapsed glioblastoma has been launched.

ATIM-36. DOSE ESCALATION TRIAL OF D2C7 IMMUNOTOXIN (D2C7-IT) ADMINISTERED INTRATUMORALLY VIA CONVECTION-ENHANCED DELIVERY (CED) FOR RECURRENT MALIGNANT GLIOMA (MG)

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BACKGROUND: D2C7-IT is a recombinant immunotoxin comprised of a dual-specific antibody fragment targeting EGFRwt and EGFRvIII and a genetically engineered form of the *Pseudomonas* exotoxin, PE38-KDEL. We report results of a phase 1 trial evaluating D2C7-IT delivered intratumorally by CED. METHODS: Eligible patients are adults with recurrent supratentorial WHO grade III or IV MG; solitary tumor; ≥4 weeks after chemotherapy, bevacizumab or study drug; adequate organ function; and KPS>70%. Planned enrollment of two patients per dose level (DL). RESULTS: As of 5/29/2018, 43 patients have been treated (2 each per DL, but for DLs 3, 6, 9, and 16). Observed dose limiting toxicities include: grade 4 seizure (n=1) on DL3, grade 3 confusion and pyramidal tract syndrome (n=1) on DL13, and grade 4 cerebral edema and grade 3 dysphasia (n=2) on DL17. Grade 2 or higher adverse events possibly related to D2C7-IT include: seizure (grade 4, n=2, grade 3, n=2, grade 2, n=4), cerebral edema (grade 4, n=1), headache (grade 3, n=4; grade 2, n=18), hemiparesis (grade 3, n=4, grade 2, n=10), dysphasia (grade 3, n=2; grade 2, n=9), confusion (grade 3, n=1; grade 2, n=5), thromboembolic event (grade 3, n=2; grade 2, n=1), fatigue (grade 2, n=6), visual field cut (grade 2, n=3), paresthesia (grade 2, n=2), stroke (grade 2, n=2), and hemineglect (grade 2, n=2); one each of grade 3 elevated ALT, urinary tract infection, fall, generalized muscle weakness, encephalopathy, and somnolence; one each of grade 2 elevated AST, papilledema, gait disturbance, intracranial hemorrhage, and urinary incontinence. Fifteen patients are alive. Two patients have partial response and are alive ≥8.2 months and ≥34 months after infusion. CONCLUSION: DL17 is above the maximal tolerated dose. Encouraging efficacy results were observed. After review of efficacy and safety data, DL13 seems the optimal dose, additional patients will be treated on DL13.

ATIM-37. SAFETY RUN-IN RESULTS OF A PHASE I/II STUDY TO EVALUATE THE SAFETY AND CLINICAL EFFICACY OF ATEZOLIZUMAB (ATEZO; aPDL1) IN COMBINATION WITH TEMOZOLOMIDE (TMZ) AND RADIATION IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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BACKGROUND: Immunotherapy strategies like PD-1/PD-L1 inhibition may work synergistically with radiation, which has been shown to increase antigen presentation and promote a pro-inflammatory tumor microenvironment. This trial evaluated the safety of concurrent atezo with radiation therapy and TMZ followed by adjuvant TMZ and atezo in newly diagnosed GBM patients. METHODS: Eligibility criteria included patients with newly diagnosed GBM age ≥ 18 yrs who have undergone only surgery. The primary objective of the phase I safety run-in was to evaluate atezo in combination with radiation and TMZ during the concurrent stage and in combination with TMZ during the adjuvant stage. RESULTS: 11 patients were accrued

in the concurrent safety run-in phase. 2 dose-limiting toxicities (DLT) were observed in 1 patient who developed both grade 3 hepatitis and pneumonitis related to atezo. 2 patients discontinued treatment during the concurrent stage due to medical complications deemed unrelated to study drug. In the 8 evaluable patients who completed the concurrent phase, no other grade 3 or 4 atezo-related toxicities were observed. 5 of the 8 evaluable patients completed the combination treatment without the need for dexamethasone. The remaining 3 of the 8 evaluable patients required no more than 4 mg daily of dexamethasone during combination treatment. All 8 patients had stable MRI findings following completion of the concurrent phase. 7 of the 8 evaluable patients proceeded with adjuvant treatment with atezo and TMZ with no grade 3 or 4 atezo-related toxicities observed to date (no. cycles range, 1–6). CONCLUSIONS: Concurrent use of atezo with radiation and TMZ was tolerable, and no new safety signals were noted. The majority of evaluable patients were able to complete the combination treatment without the need for concurrent steroid administration. The phase II component of the trial is recruiting patients (n=50) to evaluate clinical efficacy.

ATIM-38. PHASE 2 STUDY TO EVALUATE THE CLINICAL EFFICACY AND SAFETY OF MEDI4736 (DURVALUMAB, DURVA) + BEVACIZUMAB (BEV) IN BEV-NAÏVE PATIENTS WITH RECURRENT GLIOBLASTOMA (GBM)

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BACKGROUND: Durva is a human IgG1 mAb against PD-L1. Blockade of PD-1/PD-L1 has shown benefit among solid tumors; data implicate PD-1/PD-L1 signaling as a significant contributor to immunosuppression in GBM. BEV is an approved angiogenesis inhibitor for recurrent GBM; angiogenesis inhibition may promote antitumor benefit of immunotherapies. A review showed that lower dose BEV resulted in longer PFS/OS than the standard. METHODS: Ongoing Phase 2 open-label study (NCT02336165) evaluates safety and efficacy of durva (10mg/kg Q2W) in 5 GBM cohorts. Results are presented for Cohorts B2 (durva + BEV 10mg/kg Q2W) and B3 (durva +BEV 3mg/kg Q2W) in BEV-naïve recurrent GBM. Primary efficacy endpoint for Cohorts B2/B3 is 6-month progression-free survival (PFS6), by modified RANO per investigator assessment; secondary endpoints include safety/tolerability. Comparative benchmark for BEV in recurrent GBM is PFS6 of 42%. The null hypothesis (PFS6 ≤42%) was tested in Intent-to-Treat (ITT) population against the alternative hypothesis (PFS6 ≥62%). ITT includes patients receiving any dose of durva and having at least baseline and 1 post-baseline tumor assessment. Durva alone in Cohort B of this study demonstrated PFS6 of 20% (90% CI: 9.7, 33.0). RESULTS: As of 02Apr2018, 33 patients were treated in each cohort (B2, male: 54.5%, median age: 57.0 [40–74] years; B3, male: 60.6%, median age: 54.0 [23–73] years). Most common treatment-related adverse events (TRAEs, in ≥4 [12.1%] patients in either cohort): fatigue, dysphonia, increased ALT, AST, amylase, or lipase, diarrhea, hypertension, arthralgia, headache, and proteinuria. Incidences of TRAEs by maximum CTCAE grade (Gr) ≥3 for Cohorts B2/B3 were Gr3: 24.2/6.1%; Gr4: 0/6.1%; and Gr5: 0/0%. Kaplan-Meier estimate for PFS6 (n=33 each): B2, 15.2% (80% CI: 8.2, 24.0); B3, 21.1% (80% CI: 12.4, 31.4); 3 patients in each cohort showed partial response. CONCLUSIONS: The addition of durva to BEV did not improve on the outcome of durva alone.

ATIM-39. IMPROVED SURVIVAL NOTED IN GLIOBLASTOMA PATIENTS TREATED WITH ADJUVANT TLR-3 AGONIST IN SETTING OF AUTOLOGOUS LYSATE-PULSED DC VACCINATION

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Despite advances in the understanding of glioblastoma multiforme (GBM) molecular biology, genomics, and tumor microenvironment, prognosis for patients diagnosed with this disease remains dismal with standard therapies. We and others have shown utility in dendritic cell (DC) vaccination as an active immunotherapeutic treatment for these patients. In this study, we evaluated the use of autologous tumor lysate puled DC vaccine with and without adjuvant toll-like receptor (TLR) agonists. TLRs are present on dendritic cells and serve to modulate immune responses. Twenty-three patients with WHO Grade III or IV glioma were treated with three intradermal injections of autologous tumor lysate-pulsed DC on days 0, 14, and 28 followed by adjuvant placebo treatment, resiquimod (TLR-7 agonist), or poly ICLC (TLR-3 agonist). Gene expression profiling, immunohistochemistry, and mass cytometry (cyTOF) were performed on patient tumors and peripheral blood mononuclear cells. Patients that received adjuvant poly ICLC had a significantly improved median survival of 54 months over placebo (11 months) and adjuvant resiquimod (17 months) groups. Within each treatment cohort, patients with Grade III tumors had increased overall survival over Grade IV tumors. Overall, patients with MGMT methylated tumors on pathology had a median survival of 57 months, while patients with MGMT unmethylated tumors had a median survival of 19 months. Our findings suggest that adjuvant TLR-3 agonist improves outcomes with autologous lysate-pulsed DC vaccine treatment.

ATIM-40. HIGH RATE OF OBJECTIVE ANTI-TUMOR RESPONSE IN 9 PATIENTS WITH GLIOBLASTOMA AFTER VIRO-IMMUNOTHERAPY WITH ONCOLYTIC PARVOVIRUS H-1 IN COMBINATION WITH BEVACICUMAB AND PD-1 CHECKPOINT BLOCKADE

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BACKGROUND: Combination therapy is an emerging concept to improve the clinical effects of oncolytic virus based anti-cancer strategies. In a phase I/IIa trial (ParvOryx01) the oncolytic H-1 parvovirus (H-1PV) induced markers of immune activation in patients with recurrent glioblastoma. The goal of this investigation was to enhanced H-1PV efficiency by combination treatment with immune modulators, namely bevacicumab and checkpoint blockade. Methods: 9 patients (age 29 to 69 years) with primary (n=2) or recurrent (n=7) glioblastoma were treated in a compassionate use (CU) program with a combination of H-1PV followed by bevacicumab and PD-1 blockade. 7 of the patients received both intratumoral and intravenous injection of H-1PV and 2 patients only intravenous virus treatment. GMP-grade H-1 virus and medication was provided by Oryx GmbH&Co KG, Baldham, Germany) on a humanitarian basis. MRI was analyzed by an independent neuroradiologist to determine objective tumor response rate (ORR) applying RANO criteria. RESULTS: Objective tumor response was observed in 7 of 9 patients (78%). Two patients showed complete responses (22%), 5 patients had partial remissions (56%) with tumor reduction between 49% up to 94% and 2 patients progressive disease (22%). Interestingly, both patients with progressive disease showed local anti-tumor responses where virus was injected but developed new lesions. The treatment was well tolerated and lead to clinical improvement in all symptomatic patients (n=5). CONCLUSION: H-1PV based viro-immunotherapy lead to ORR in 78% of glioblastoma patients. This is a much higher response rate than reported for treatment with either bevacizumab or checkpoint blockade and it supports further systematic clinical development of this novel concept for malignant glioma therapy.

ATIM-41. PHASE II TRIAL OF A SURVIVIN VACCINE (SurVaxM) FOR NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: Survivin is an anti-apoptotic protein that is highly expressed in glioblastoma (GBM). We conducted a single-arm, multi-center phase II trial in newly diagnosed GBM (nGBM) to determine 6-month progression-free survival (PFS-6), 12-month overall survival (OS-12) and immunologic response in patients treated with surgery, chemoradiation, adjuvant temozolomide (TMZ) and survivin-targeted immunization. METHODS: Patients with nGBM who hadwith HLA-A*02, -A*03, -A*11 and -A*24 haplotypes and, Karnofsky performance status ≥70 were included. Following craniotomy (3 residual contrast enhancement) and chemoradiation, patients received 4 prime-boost doses of SurVaxM (500

mcg) every 2 weeks, followed by adjuvant TMZ and maintenance SurVaxM every 12 weeks until progression. Immunogenicity of SurVaxM was assessed by measuring anti-survivin antibody levels and survivin-specific CD8+ T-cells using multimers. RESULTS: Sixty-three patients, median age 60 years (range, 20–82), including 38 males were treated. Survivin expression was detectable in all patients (1–40% ([median = 12%]) of tumor cells by immunohistochemistry). The vaccine was highly immunogenic and produced survivin-specific CD8+ T-cells and antibody (IgG) titers. The regimen was well-tolerated and immunogen-related adverse events were mild with no regimen limiting toxicity attributable to SurVaxM. PFS-6 was 96.7% (C.I. = 87.6%- 99.1%) and OS-12 was 94.2% (C.I. =83.0%-98.1%) as measured from diagnosis. In MGMT methylated, PFS6 of 96.9% (C.I. = 99.6% to 79.8%); OS12 of 98.1% (C.I. = 99.0% to 97.2%) and in unmethylated tumors, PFS6 of 96.6% (C.I. = 99.6% to 78%); OS12 of 88.9% (C.I. = 99% to 77.2%) was observed. Methylated patients with higher survivin levels had significantly better PFS-6 than those with low survivin levels (r = 0.4). CONCLUSIONS: SurVaxM is safe and a promising adjunct therapy in nGBM. Compared to historical matched controls, addition of SurVaxM improved PFS-6 and OS-12 in nGBM. Patients with poor prognostic factors (unmethylated MGMT, higher survivin levels) treated with SurVaxM achieved better survival than expected.

ATIM-42. SAFETY AND EFFICACY OF AUTOLOGOUS DENDRITIC CELLS/TUMOR CELL ANTIGEN ADJUVANT THERAPY OF GLIOBLASTOMA MULTIFORME: RESULTS OF 59 CASES

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In our translational research, an immunotherapeutic, ADCTA-G, has been developed to emphasize autologous tumor antigens, DC antigen processing/presentation, enhanced tumor immunogenicity and CTL induction. Two clinical trials, Phase I/II [2003-2005Taiwan DOH/MA 0910072504] and phase II [2005–2016 NIH NCT02772094], "Dendritic Cell(DC)-Based Tumor Vaccine Adjuvant Immunotherapy of Human Glioblastoma Multiforme", respectively enrolled 17 and 42 WHO Grade-IV glioblastomas. Every patient received peripheral blood apheresis for PBMNCs. Monocytes were used for derivation of 3-7x108 phagocytic dendritic cells (iDC). Autologous glioma cells grown out of surgical tumor specimen were irradiated and co-cultivated 1 to 2:1 with iDC to make a ADCTA-G lot. After surgical tumor de-bulking, 10 vaccinations were given, each with 2-5x107 mature DC from the ADCTA lot, in a [4x biweekly and 6x monthly] scheduled s.c. injection in both axillar regions. The follow-up period has been 15 years. Primary endpoint is the overall survival (OS); phenotypes of tumor infiltrating T cells and tumor IDH mutation were also analyzed for long-term survivors. The ADCTA-G inoculations were tolerated well, and curtailed the grade-3/4 lymphopenia adverse effect of the temozolomide CCRT. The median OS is 22.9 months for the total 59 grade IV patients in the two trials. The median OS is 21.8 months for the 44 newly diagnosed patients and 28.1 months for the 15 recurrent patients; the difference is insignificant statistically. In this study, vaccinations were initiated early in the recurrent GBM patients while the newly diagnosed GBM patients had to wait till after external radiation therapy, leading to a comparable OS benefit of the ADCTA vaccination. Also, we demonstrated in vitro the earlier vaccinations in the recurrent decrease CD133+ tumor stem-like cells. In addition, the CD8(+) cells were susceptible to ionizing radiation. A phase 3 open-labelled randomized study will be initiated to improve the survival of this aggressive malignancy.

ADULT CLINICAL TRIALS - NON-IMMUNOLOGIC

ACTR-01. SAFETY ANALYSES OF TUMOR TREATING FIELDS IN COMBINATION WITH LOMUSTINE IN THE EF14 PHASE 3 CLINICAL STUDY

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INTRODUCTION: Tumor Treating Fields (TTFields) are low intensity (1-3V/cm), intermediate frequency (100–300 KHz) alternating electrical fields approved for glioblastoma (GBM). In the EF-14 phase 3 study, TTFields showed a significant overall and progression-free survival benefit for patients with newly diagnosed GBM in combination with Temozolomide (TMZ). TTFields were not associated with systemic toxicity. At recurrence, patients continued TTFields with second line therapies. We analyzed the safety and feasibility of TTFields + lomustine (CCNU) combination. Methods: Patients in the EF-14 trial received TTFields until second progression, or for 24 months. Change in chemotherapy regimen was allowed in both groups after tumor progression. We compared patients who received lomustine as second-line chemotherapy in combination with TTFields (n=134) to patients who received lomustine as monotherapy after first progression (n=39). We compared baseline characteristics and the adverse event profile between the two groups. RESULTS: Baseline characteristics were well balanced; there were less female patients in the lomustine only group (7.7% vs. 22.4%). Median age in the TTFields/lomustine group was 55.5 years (29–83) versus 50.0 years (19–71) for lomustine alone. The addition of TTFields to lomustine therapy was not associated with any significant increase in rates of systemic adverse events compared to lomustine therapy alone (number of patients with ≥ 1 SAE 30 % vs. 31 %) and the distribution, severity and overall incidence of adverse events were not statistically different in patients in the two treatment groups. CONCLUSION: The combination of TTFields and lomustine is safe and feasible. This analysis emphasizes again the strong safety profile of TTFields and the high potential of combining TTFields with other therapy modalities. This data is especially important in light of the recently presented promising data from a small randomized trial that tested the combination of lomustine plus TMZ in newly diagnosed (MGMT promotor-methylated only) GBM patients.

ACTR-02. NRG ONCOLOGY/RTOG 0424: LONG-TERM RESULTS OF A PHASE II STUDY OF TEMOZOLOMIDE-BASED CHEMORADIOTHERAPY REGIMEN FOR HIGH-RISK LOW-GRADE GLIOMAS

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PURPOSE: To report the long-term outcomes and MGMT analysis of temozolomide (TMZ) and radiotherapy (RT) in a high-risk low-grade gliomas (LGG) population. PATIENTS/ METHODS: For this single-arm phase II study, LGG patients with ≥3 risk factors (age ≥40, astrocytoma, bi-hemispheric tumor, size ≥6 cm or preoperative neurologic function status >1) received RT (54 Gy/30 fractions) with TMZ and up to 12 cycles of post-RT TMZ. The primary endpoint was overall survival (OS) at 3 years after registration. A one-sided Z-test was used to test the hazard rate based on the observed 3-year OS rate versus a prespecified historical control from the EORTC high-risk LGG population. Secondary endpoints included progression-free survival (PFS), and the association of survival outcomes with MGMT methylation status, for which the MGMT-STP27 prediction model was used based on 450k data. The initial report of this study was published in 2015, when the results of the MGMT analysis were unavailable. RESULTS: The study accrued 129 analyzable patients. The median follow-up for surviving patients was 9 years (range: 0.4–11.8), 4 years longer than previously reported. The 3-year OS rate was 73.5% (95% CI: 65.8–81.1%), superior to the historical control of 54% (p

ACTR-03. A FEASIBILITY TRIAL OF THE MODIFIED ATKINS DIET AND BEVACIZUMAB FOR RECURRENT GLIOBLASTOMA

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BACKGROUND: We report results of a feasibility trial of the modified Atkins diet (MAD), a form of ketogenic diet (KD), combined with bevacizumab (Bev) for recurrent GBM. The rationale for a KD to treat GBM includes the reduction of glucose availability to this highly glycolytic tumor, reduction of a variety of GBM growth signaling pathways, and increase in tumor-reactive immune responses. Clinical and laboratory data indicate the combination of KD and Bev is more effective than either

treatment alone (Reiger et al 2014). Because adherence to the MAD and Bev in patients with GBM cannot be predicted, we performed a measure of treatment compliance before proceeding to an efficacy trial. METHODS: Feasibility study in 12 Bev naïve recurrent GBM patients. Bev was administered in standard doses biweekly. The primary objective was to document compliance with MAD in at least 60% of the patients (7/12) at least 80% of the time at 12 weeks. Secondary objectives included adverse events, tumor response, PFS, OS. Monitoring of MAD biologic effects included serum glucose and urine ketones daily and serum B-hydroxybutyrate biweekly. Compliance was determined by review of daily food diary and ketone levels. RESULTS: 8/12 patients completed the trial at 12 weeks and all were compliant with diet and all achieved ketosis. One additional patient continues on study at 48 days. Three patients were removed from study: grade 3 hypertension related to Bev, grade 3 seizures related to tumor, tumor progression at 71 days. CONCLUSION: We document the feasibility of the MAD and Bev in recurrent GBM. We will present correlations of treatment outcome with serum levels of ketosis, glucose, insulin, amino acids and with tumor MCT4 expression and IDH1 mutation status. Funding: Ohio Clinical Trials Collaborative, Blast GBM, Sally S. Morley Memorial Glioblastoma Treatment Fund, Nutricia

ACTR-06. INITIAL RESULTS OF A PHASE I STUDY OF PROCASPASE ACTIVATING CCOMPOUND-1 (PAC-1) IN COMBINATION WITH TEMOZOLOMIDE FOR RECURRENT MALIGNANT GLIOMA

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Disruption of the intrinsic and extrinsic apoptotic pathways is a hallmark of neoplasia. Conversion of procaspase-3 to caspase-3 is a key reaction, as both pathways converge at this point. Procaspase activating compound -1 (PAC-1) catalyzes conversion of procaspase-3 to caspase-3 and induces apoptosis in tumor cells. Glioblastoma (GBM) is among the tumors that have high concentrations of procaspase-3 and low levels of caspase-3. PAC-1 has anti-tumor activity in several glioma cell lines *in vitro*. PAC-1 crosses the blood brain barrier and its addition to alkylating chemotherapy augments anti-tumor responses in *in vivo* rodent models and spontaneous canine gliomas. Taken together, these findings suggest PAC-1's potential in glioma therapy. This dose-escalation phase I study to assesses the maximum tolerated dose (MTD) of PAC-1 administered daily for 21 days with TMZ, 150 mg/m^2 for 5 days of each 28 day cycle in subjects with recurrent anaplastic astrocytoma (AA) or GBM. RANO criteria are used to assess response. A modified Fibonacci 3 + 3 design is used, expanding to 9 subjects at the MTD. Pharmacokinetics (PK) is assessed in each subject during cycle one. Secondary endpoints include pharmacodynamics and correlation of activity with procaspase-3 levels in tumor tissue. Neurologic toxicity, including cognitive function, is closely monitored throughout the trial. 6 subjects (all GBM) enrolled at the first dose level, 375 mg PAC-1/day. The PAC-1cycles administered ranged from 1–6 (mean = 3.2). All subjects left the study due to tumor progression. The best radiographic response was stable disease. The first cohort was expanded from 3 to 6 due to CTCAE grade 4 hepatotoxicity that resolved with dose reductions in both study drugs. An update of toxicity data and results of PK and tumor procaspase levels will be discussed.

ACTR-07. COMPLETE, DURABLE RESPONSE OF A RECURRENT UNRESECTABLE GRADE III GLIOMA TO A REPURPOSED DRUG REGIMEN

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Some non-oncology drugs can be repurposed as adjuvants to traditional cytotoxic chemotherapies. Cancer after all uses dysregulated and pathologically activated, but otherwise normal, physiological growth factors, anti-apoptosis pathways and migration mechanisms. We manipulate these pathways every day in non-cancer medicine so it is straightforward to use these non-cancer related medicines to address dysregulated growth systems in cancer. We report here a 28 y/o man diagnosed in May 2011 with Grade II/III Astrocytoma, 1p/19q intact, IDH1 R132H wild type. Subtotal resection left mass-like FLAIR involving right internal capsule along with patchy enhancement. After 1 year of 5-day temozolomide, 20 mg bid, ending in Sept 2012, he was followed with serial MRI scans. After progression of enhancing tumor in May 2015, repeat biopsy demonstrated anaplastic (gemistocytic) astrocytoma. Radiation/temozolomide/naltrexone was followed by adjuvant therapy temozolomide 20 mg BID, celecoxib 200 mg daily, naltrexone 25 mg daily, and levetiracetam 1500 mg BID for seizure control. In June 2017 new enhancement appeared deep to the surgical cavity. On patient's initiative, addition of valproic acid 500 mg BID to current therapy. He has tolerated this ongoing treatment with no toxicities and remained clinically asymptomatic, continuing to work full time. Over the interval (mid-2017 to summer 2018), the enhancing components of the tumor, which had persisted for years, have completely resolved. CONCLUSION: Repurposed drug cocktails, which can simultaneously and specifically target multiple tumor pathways remain a largely untested treatment for patients who face limited proven options. We report a patient who continues to do well in the face of an inoperable, poor molecular profile recurrent tumor with a inexpensive drug regimen that has yielded no toxicity. Repurposing non-oncology drugs to augment traditional cytotoxic chemotherapy hold great promise.

ACTR-08. A PHASE II STUDY OF APATINIB PLUS TEMOZOLOMIDE IN ADULTS WITH REFRACTORY RECURRENT HIGH-GRADE GLIOMAS

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BACKGROUND: There is no standard therapy for refractory recurrent high-grade gliomas. We assessed the efficacy and safety of apatinib, a new oral small-molecule tyrosine kinase inhibitor targeted vascular endothelial growth factor receptor 2, combined with temozolomide (TMZ) in patients with refractory recurrent high grade gliomas. METHOD: This was a single-arm phase prospective clinical trial. Thirty three patients with recurrent high-grade gliomas were enrolled from April 2016 to March 2018. They received oral apatinib (500mg qd) in combination with TMZ. TMZ was administrated at 200 mg/m^2/d according to standard 5/28 days regimen for the patients who had not received TMZ before, and that patients who had experienced relapse from the standard 5/28 TMZ regimen, received continuous daily TMZ (50 mg/m^2/d). After 12 cycles, the patients continued to take apatinib as maintenance until progression. The primary endpoint was a 6-month progression-free survival (PFS) rate. RESULTS: The 6-month PFS for glioblastoma (GBM) was 47.8% and 58.3% for anaplastic gliomas (AGs). The median PFS was 4.9 months for GBM and 6.3 months for AGs. The overall survival (OS) at 1 year was 36.2% for GBM and 17.9% for AGs. The median OS for GBM was 8.3 months and 10.6 months for AGs. The differences in PFS (P=0.77) or OS (P=0.70) between GBM and AGs did not reach significance. Five (25%) out of 20 patients with GBM demonstrated partial (3/20) or complete (2/20) radiographic response to treatment and 10/20 (50%) remained stable. Four (50%) out of 8 patients with AGs showed partial (3/8) or minor (1/8) response to treatment and 3/8 (37.5%) remained stable. The most common grade 3 to 4 nonhematologic toxicities were hypertension, hand-foot syndrome, proteinuria and elevated transaminase, which were acceptable. CONCLUSIONS: These data show that apatinib plus TMZ is effective and tolerable in patients with refractory recurrent high-grade gliomas.

ACTR-09. TARGETING MYELOID DERIVED SUPPRESSOR CELLS: PHASE 0/1 TRIAL OF LOW DOSE CAPECITABINE + BEVACIZUMAB IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) creates an immunosuppressive environment that allows tumor growth. Myeloid derived suppressor cells (MDSCs) mediate immunosuppression in GBMs. MDSCs are up-regulated in the blood of GBM patients. We have developed a novel strategy to target GBM immunosuppression using low dose 5-fluorouracil (5-FU) to target MDSCs. Marked MDSC depletion occurs at 5-FU doses in mice equivalent to <10% of the normal human dosing. Goal: proof of concept that MDSC suppression is feasible in GBM patients with low-dose capecitabine [cap], (oral 5-FU analogue). METHODS: Eligibility: Recurrent GBM in need of surgical resection; no prior cap or bevacizumab. Cohorts of 3–6 patients receive low-dose cap 150 mg/m2/d (dose level 1) for 7 days pre-surgery. Post-op, patients resume cap for one cycle after which bev is added. Concentrations of blood MDSCs, immune cells, and relevant secreted factors are measured at baseline; pre- and post-op; and after the addition of bev. Tumors are assayed for MDSCs and glioma stem-like cells (GSCs). Primary endpoint: MDSC and T-regulatory cell (T-reg) reduction after cap. RESULTS: Seven patients have enrolled to date. In all patients MDSCs rose initially after resection (max rise 18% over baseline) and subsequently began to fall after cycle 2 (max reduction 6%). T-regs fell slightly (0–12%) in 5 of 7 patients. Cytotoxic T-cell (CTL) concentrations rose significantly (max 93%). CD3+ T-cells fell in 5 of 7 patients (max reduction 52%). Four of 7 (71%) of patients reached PFS6. Median PFS-6 months (2–14 mos). Median overall survival 10 months (5–16). Treatment-related SAEs: grade

3 dyspnea; grade 2 hemorrhage, non-neutropenic fever; and grade 1 hand-foot. CONCLUSIONS: Low-dose capecitabine is associated with a modest reduction in MDSCs and T-regs and a significant increase in CTLs. Toxicity has been manageable. Four of 7 evaluable patients have reached 6 months free of progression. Dose escalation continues.

ACTR-10. A RANDOMIZED, PHASE I/II TRIAL OF IXAZOMIB IN COMBINATION WITH STANDARD THERAPY FOR UPFRONT TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED MGMT METHYLATED GLIOBLASTOMA (GBM) STUDY DESIGN

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OBJECTIVE: To investigate the toxicity, tolerability and efficacy of the combination of ixazomib and standard chemoradiation therapy for newly diagnosed MGMT methylated GBM patients. BACKGROUND: GBM is the most aggressive primary malignant brain tumor. Standard therapy with temozolomide and radiotherapy after the surgery offers limited overall survival (OS). The median OS in patients with MGMT methylation is still less than 2 years. Our recent phase II clinical trial found that the addition of bortezomib, a proteasome inhibitor, to the standard therapy, offered mild survival benefit (19 months) for the entire group of GBM patients. However, significant improvement of OS and progression free survival (PFS) were found in the MGMT methylated patients when compared to MGMT unmethylated patients (OS: 49.4 vs 15.6 months, p=0.0002; PFS: 24.7 vs 5.1 months, p=0.00004) [Kong et al. Int J Radiation Oncology Biol Phys 2018]. Ixazomib is a newer generation of proteasome inhibitor and has demonstrated similar selectivity and potency to bortezomib in biochemical and cell-based assays. While bortezomib can be administered via injection, ixazomib is taken orally, which is more convenient. HYPOTHESIS: Adding ixazomib to standard therapy improve the survival of the patients with newly diagnosed MGMT-methylated GBM compared to standard therapy. STUDY DESIGN: This is a randomized, active controlled, open label phase I/II study of Ixazomib plus standard therapy versus standard therapy. Primary and secondary endpoints are PFS, 12, 24, 36 and 48 month survival rates and response duration. The study consists of two parts: In part I, the maximum tolerated dose (MTD) is decided by using 3 + 3 design with dose limiting toxicity (DLT) method. In part II, randomize the patients to the combination therapy or the standard therapy arm. Safety will be assessed by CTCAE V4.03. We will use Kaplan-Meier estimates for survival data and a stratified log-rank test for the randomization strata.

ACTR-12. PRELIMINARY SAFETY AND EFFICACY OF A PHASE II TRIAL OF 18F-DOPA PET-GUIDED, DOSE-ESCALATED RADIOTHERAPY IN THE TREATMENT OF GLIOBLASTOMA

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BACKGROUND: 18F-DOPA-PET thresholds reliably delineate areas of high-grade astrocytoma not otherwise recognized with standard MRI and may more accurately identify regions of aggressive, high-density disease. Herein we report the preliminary safety and feasibility data from an ongoing phase II study (MC1374; R01CA178200) evaluating 18F-DOPA-PET guided-dose-escalated radiotherapy for glioblastoma. METHODS: Newly diagnosed glioblastoma patients without contra-indications to 18F-DOPA-PET are eligible for study enrollment. Target volumes include: CTV51Gy=T1-gadolinium contrast-enhancing (T1-CE) disease, T2 FLAIR signal abnormality, and low-grade 18F-DOPA-PET uptake, +1cm; CTV60Gy=T1-CE and high-grade 18F-DOPA-PET uptake, +1cm; and CTV76Gy=T1-CE and high-grade 18F-DOPA-PET disease without expansion all given in 30 fractions simultaneously. Patients are followed with 18F-DOPA-PET in addition to standard clinical follow-up. Safety stopping rule specifies that after 10 or more patients have been enrolled, if more than 10% experience any of the following adverse events considered to be at least possibly related to treatment, enrollment will be suspended: Grade 3 or 4 irreversible CNS toxicity, Grade 4 non-hematologic, non-CNS toxicity, any Grade 5 toxicity. Futility analysis (and primary study aim) is powered to consider a success to be an MGMT-unmethylated patient who is without progression within 6 months from the time of craniotomy. If 16 or more successes are observed in the first 25 evaluable patients study will continue. RESULTS: 77 patients have been accrued since December 2013 with 68 evaluable for toxicity. Grade 3 CNS necrosis was noted in 3 (4.4%) patients; 2 additional patients developed symptoms that resolved in the subsequent cycle so did not count towards stopping rule. Other grade 3+ toxicities include: 1 patient with pre-existing vision dysfunction had Grade 4 optic nerve dysfunction; 2 Grade 4 hematologic events and 1 Grade 5 event(sepsis) due to temozolamide-induced cytopenias. CONCLUSION: 18F-DOPA-PET -guided dose escalation appears reasonably safe and tolerable in patients with high-grade glioma.

ACTR-13. A BAYESIAN ADAPTIVE RANDOMIZED PHASE II TRIAL OF BEVACIZUMAB VERSUS BEVACIZUMAB PLUS VORINOSTAT IN ADULTS WITH RECURRENT GLIOBLASTOMA FINAL RESULTS

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BACKGROUND: Bevacizumab improves outcome and reduces symptoms in patients with recurrent glioblastoma (GBM). However, GBMs develop adaptive resistance to bevacizumab- mediated angiogenesis inhibition resulting in tumor recurrence. We hypothesized that vorinostat, a histone deacetylase (HDAC) inhibitor, with pleotropic antiangiogenic effects, would delay emergence of resistance to bevacizumab therapy and improve clinical outcome. METHODS: In this multicenter phase II trial utilizing a novel Bayesian design, patients with recurrent glioblastoma were adaptively randomized to bevacizumab alone or bevacizumab+vorinostat based on a primary endpoint of progression-free survival (PFS) such that patients had a higher likelihood of receiving the more efficacious treatment. Secondary end points were overall survival (OS) and quality of life assessment (MDASI-BT). Eligible patients were adults (≥ 18 yrs) with histologically confirmed GBMs recurrent after prior radiation and temozolomide therapy, adequate organ function, KPS≥ 60, and no prior bevacizumab/HDAC inhibitors. RESULTS: Ninety patients (bevacizumab+vorinostat:49, bevacizumab:41) were enrolled and 74 were evaluable for PFS (bevacizumab+vorinostat:44, bevacizumab:30). Grade 3 or greater toxicities in 85 evaluable patients included hypertension (n=37), neurological changes (n=2), anorexia (n=2), infections (n=9), wound dehiscence (n=2), DVT/PE (n=2), and colonic perforation (n=1). There was one treatment-related death due to pulmonary embolism. Upon multivariate analysis for bevacizumab+vorinostat vs bevacizumab, median PFS (3.7 vs. 3.9 months, p=0.94, HR 0.63 [95% CI 0.38, 1.06, p=0.08]) or median OS (7.8 vs. 9.3 months, p=0.64, HR 0.93 [95% CI 0.5, 1.6, p=0.79]) were not significantly different between the two arms. Ongoing analyses of patient reported outcomes (MDASI-BT) and plasma biomarkers will be reported. CONCLUSIONS: Combining bevacizumab with vorinostat did not result in improved PFS or OS compared with bevacizumab alone in patients with recurrent GBM. This trial is the first to test a Bayesian PFS-based adaptive randomized design in patients with primary brain tumors and demonstrates the feasibility of using adaptive randomization in a multicenter setting.

ACTR-14. PHASE I STUDY OF AZD1775 WITH RADIATION THERAPY (RT) AND TEMOZOLOMIDE (TMZ) IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM) AND EVALUATION OF INTRATUMORAL DRUG DISTRIBUTION (IDD) IN PATIENTS WITH RECURRENT GBM

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AZD1775 is an oral small molecular inhibitor of the G2/M checkpoint regulator Wee1. The Adult Brain Tumor Consortium 1202 trial (NCT01849146) is a phase I, open label, multicenter dose-finding study of AZD1775 in combination with standard RT and TMZ followed by an IDD study for patients undergoing surgery for recurrent GBM. Dose of AZD1775 was increased in a 3 + 3 design M-F during concurrent RT/TMZ and x 5d/28d cycle with adjuvant TMZ in separate cohorts. A combination cohort with both concurrent and adjuvant AZD1775 at MTD and analysis of PK/PD and IDD at MTD in patients undergoing surgery for recurrent GBM followed. MTD was 200 mg for concurrent with 2/6 patients experiencing DLTs (grade 4 neutropenia, grade 3 ALT elevation). MTD for the adjuvant cohort was 425 mg with 1/6 patients experiencing DLT (grade 4 decrease in ANC). 6/12 patients experienced DLTs when cohorts were combined, however, five during the concurrent phase. Three patients had grade ≥3 ALT/AST elevation, one had grade 3 afib, and one had grade 4 neutropenia/thrombocytopenia, grade 3 dehydration/fatigue/muscle weakness. A sixth patient had grade 4 neutropenia in the first adjuvant cycle. Following amendment, an additional 6 patients were enrolled with 150 mg (concurrent) and 425 mg (adjuvant) combination and are in the observation period with one DLT currently. Drug concentration in contrast enhancing and non-enhancing brain tumor was 4–8 x and 0.5–2.6 x greater than plasma, respectively for patients on IDD portion. CONCLUSIONS: AZD1775 in combination with RT/TMZ at 200 mg qd M-F with concurrent RT/TMZ and 425 mg qd x 5d/28d cycle in combination with adjuvant TMZ had unacceptable DLT rate in the concurrent phase. A cohort with 150 mg concurrent/425 mg adjuvant has competed accrual with acceptable rates of toxicity currently in observation. AZD1775 has good penetration to non-enhancing and enhancing tumor areas.

ACTR-15. SAFETY AND PRELIMINARY ACTIVITY OF PT2385, A FIRST-IN-CLASS HIF2-ALPHA INHIBITOR, PLANNED INTERIM ANALYSIS OF AN OPEN LABEL, SINGLE-ARM PHASE II STUDY IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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BACKGROUND: Hypoxia inducible factor 2-alpha (HIF2a) is a stress response transcription factor that mediates the cellular response to hypoxia. HIF2a is an underexplored target in glioma. PT2385 is a first-in-class oral HIF2a inhibitor with favorable blood-brain barrier penetrating properties and *in vivo* single-agent activity against glioblastoma (GBM). METHODS: A single-arm open-label phase II study of adults with bevacizumab-naïve first recurrence of GBM following chemoradiation with measurable disease was conducted within the Adult Brain Tumor Consortium. PT2385 was administered at the recommended phase II dose (800 mg b.i.d.). The primary study outcome is objective radiographic response (CR+PR). Secondary objectives are safety, overall survival, and progression-free survival. Patients at selected study sites underwent pH-weighted amine-CEST MRI imaging to quantify tumor acidity at baseline and explore associations with drug response. Results of planned interim analysis are presented. RESULTS: 24 patients were enrolled; mean age 61 ± 11 years, 63% male, 92% white, median KPS 80. MGMT promoter was methylated in 46%, unmethylated in 50%, and indeterminate in 1 patient. Prior surgery included biopsy (8%), subtotal (38%) and gross total resection (54%). To date, 21 patients have progressed at a median of 7.7 weeks (95%CI 4.66–12.3 weeks). No objective radiographic responses have been observed. Three patients continue on treatment at a median of 19 weeks (95%CI 12–19.1). The drug was well tolerated with expected side effect profile. Common Grade 1–2 drug-related adverse events were anemia, dyspnea, and thrombocytopenia. Grade 3–4 drug-related adverse events included hypoxia (n=2, 8%), anemia (n=1, 4%), and hypophosphatemia (n=1, 4%). At baseline, pH-weighted MRI showed high levels of acidity and intratumoral heterogeneity. PK and PD data are forthcoming. CONCLUSIONS: Results of this planned interim analysis suggest that single-agent PT2385 has acceptable safety but minimal activity in glioblastoma patients after first recurrence. Ongoing analysis will explore patterns of progression and correlate these with tumor acidity measurements.

ACTR-16. PERIPHERAL BLOOD CD4+ MONONUCLEAR CELL FRACTIONS ARE ASSOCIATED WITH OVERALL SURVIVAL AT FIRST RECURRENCE OF IDH-WILDTYPE GLIOBLASTOMA AFTER STANDARD CHEMORADIOTHERAPY: SECONDARY ANALYSES OF THE PHASE II DIRECTOR TRIAL

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The alkylating agent temozolomide prolongs survival of glioblastoma patients through induction of futile DNA mismatch repair in cancer cells. Whether systemic temozolomide effects on the immune system affect outcome has not been studied in detail. To address this question, we analyzed peripheral blood mononuclear cells (PBMC) of N=52 clinically well-annotated patients with recurrent, isocitrate dehydrogenase (IDH)-wildtype glioblastoma and of N=21 healthy donors by 11-color flow cytometry. Patients were treated within the randomized phase II trial DIRECTOR, which explored the efficacy of two dose-intensified temozolomide regimens at first recurrence of glioblastoma after standard chemoradiotherapy with first-line temozolomide. There were no efficacy differences between both dose-intensified temozolomide schedules in this trial. Unsupervised clustering of flow cytometry annotations identified two patient clusters, which differed in CD4+ T-cell fractions, but not with respect to CD8+ T-cells, CD4+;CD25+;FoxP3+ regulatory T-cells, B-cells or monocytes. All control samples clustered with the $CD4_{high}$ cluster. Patients in both clusters did not differ by age, gender, O6-methylguanine-DNA-methyl-transferase (*MGMT*) gene promoter methylation, tumor volume, Karnofsky performance score (KPS), number of first-line temozolomide cycles or steroid use. Progression-free survival was similar ($CD4_{high}$ *vs* $CD4_{low}$2.1 *vs* 2.4 months, p=0.19), whereas overall survival was longer in the $CD4_{high}$ cluster of patients (12.7 *vs*8.7 months, p= 0.004). In a multivariate Cox model of overall survival that controlled for established prognostic factors, we found associations with overall survival for KPS (p=0.019), high CD4+ fractions (p=0.052) with relevant interactions with cluster assignment (p=0.058), residual tumor at study entry (p=0.068) and *MGMT* promoter methylation (p=0.072), but not age (p=0.96) or steroid use at study entry (p=0.32). There were no interactions of cluster assignment or CD4+ fractions with steroid use in this model. We conclude that temozolomide-associated CD4+ T-cell depletion may have unfavorable effects on the survival of glioblastoma patients, a finding that warrants further exploration.

ACTR-17. EVOPHOSPHAMIDE (TH-302) FOR RECURRENT GBM FOLLOWING BEVACIZUMAB FAILURE, FINAL RESULTS OF A MULTICENTER PHASE II STUDY

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INTRODUCTION: Evophosphamide is a hypoxia-activated prodrug that, when activated in hypoxic conditions, (<0.5% O2), releases the bis-alkylating agent bromo-isophosphoramide mustard (Br-IPM). Our prior phase 1 study in recurrent GBM (rGBM) with dose expansion showed preliminary activity with a 24% objective response rate and a 26% PFS rate at 4 months. METHODS: A multicenter, single-arm, two-stage prospective study, non-blinded with combination therapy with bevacizumab at 10 mg/kg intravenously (IV) every 2 weeks and TH-302 at 670 mg/m2 IV every 2 weeks, in 6 week cycles, until disease progression. The primary endpoint was progression free survival at 4 months (PFS4). Patients underwent baseline assessment for hypoxic burden by 18F-misonidazole PET, dynamic susceptibility contrast (DSC) perfusion imaging, and serum sampling for biomarker analysis. RESULTS: 36 patients received study drug. Treatment was well tolerated, with adverse events as expected and the most common toxicity rash along the perineum. The PFS4 rate was 25% which met the primary endpoint and compares favorably with historical controls (10%). Biomarker analysis revealed progression to be correlated with Tmax on DSC perfusion

(HR=0.8, 95% CI 0.66 to 9.96, p=0.02), ratio of enhancement on anatomic MRI Ratio (HR=9.37, 95% CI 1.18 to 74.5, p=0.03) and survival was most closely correlated with hypoxic volume on FMISO PET (HR=1.02, 95% CI 1 to 1.04, p=0.01). CONCLUSION: Evofosfamide has modest activity in bevacizumab refractory glioblastoma, with progression and survival correlated with radiographic features at baseline.

ACTR-18. PHASE II TRIAL OF TEMOZOLOMIDE AND TRC 102, BASE EXCISION REPAIR INHIBITOR, IN BEVACIZUMAB NAÏVE GLIOBLASTOMA AT FIRST RECURRENCE

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BACKGROUND: Temozolomide forms O^6-methylguanine (O^6mG), 7-methylguanine (N^7mG), and 3- methyladenine (N^3mA) DNA adducts. The O^6mG DNA adduct is repaired by MGMT. N^7mG and N^3mA DNA adducts are removed by the base excision repair (BER) pathway. TRC-102 is a BER inhibitor that binds to the apurinic site created through the action of the glycosylase. METHODS: A phase II study of adult patients with bevacizumab-naïve first recurrence of glioblastoma after radiation and temozolomide was performed in the Adult Brain Tumor Consortium. Temozolomide was administered at 150 mg/ m^2 and oral TRC-102 at 150 mg daily, days 1–5 every 4 weeks. Primary objective was efficacy measured by objective radiographic response rate (RR= CR+PR). Secondary objectives included safety and PFS-6. Exploratory objectives were to assess treatment efficacy with tumor expression of N-methylpurine DNA glycosylase (MPG), a BER protein, and MGMT status with RR, PFS, and OS. The study was designed to test the hypothesis that combination therapy would achieve a RR of 30%. RESULTS: Nineteen patients were enrolled in the first stage. Median age was 60 years (range: 48–76), 53% females, median KPS was 80 (range: 70–90). Median cycles of treatment was 2 (range: 1–12). No responses were observed. Median OS was 11.0 months (95% CI: 8–18 months), median PFS was 2.0 months (95%CI: 1.8–3.6 months). PFS-6 rate was 10.5 % (2/19). The combination was safe; two grade 3–4 toxicities included lymphopenia, thrombocytopenia. MGMT promoter was unmethylated in all patients. MPG staining was negative in six, 1+ in five and 2+ in three patients. PFS of 11 + months in two patients was associated with MPG expression. CONCLUSIONS: TRC 102 and temozolomide has acceptable safety but did not meet the primary endpoint of response. Tissue correlates will be presented. The study was terminated early and the combination will not be tested in bevacizumab refractory patients.

ACTR-19. A MULTICENTER PILOT PHASE II STUDY OF CONTINUING TMZ WITH THE ADDITION OF DISULFIRAM AND COPPER FOR REFRACTORY GLIOBLASTOMA

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BACKGROUND: Preclinical studies have suggested promising activity for the combination of disulfiram and copper (DSF/Cu) against glioblastoma (GBM) including re-sensitization to temozolomide (TMZ). A previous phase I study demonstrated the safety of combining DSF/Cu with adjuvant TMZ for newly diagnosed GBM. This pilot phase II study aimed to estimate the potential effectiveness of DSF/Cu to re-sensitize recurrent GBM to TMZ. METHOD: This open-label, single-arm phase II study treated recurrent TMZ-refractory GBM patients with TMZ 150mg/m^2 on days 1–5 of every 28-day cycle with concurrent daily DSF 80mg TID and Cu 1.5mg TID. Eligible patients must have progressed after standard chemoradiotherapy and within 3 months of the last dose of TMZ. Known IDH-mutant or secondary GBMs were excluded. The primary endpoint was objective response rate (ORR), and the secondary endpoints included progression-free survival (PFS), overall survival (OS), clinical benefit (stable disease for at least 6 months), and safety. Evaluable patients must have received at least 28 days of DSF/Cu unless stopped due to progression, toxicity, or death. RESULTS: From March 2017 to January 2018, 23 TMZ-refractory GBM patients were enrolled across seven centers in the United States, and 22 patients were evaluable. The median DSF/Cu duration was 48 days (range: 12–246 days). After a median follow-up of 4.4 months, there were no objective responses, with 6-month PFS of 14% and 6-month OS of 55%. Among 17 patients who had at least 28 days of DSF/Cu, 3 patients (18%) had clinical benefit. Grade 3 toxicities that were possibly related to DFS/Cu included fatigue, headache, anxiety, and elevated alanine transaminase (5% for each). CONCLUSION: Addition of DSF/Cu to TMZ for TMZ-refractory GBM yielded minimal ORR but demonstrated clinical benefit for a subset of patients. DSF/Cu may have modest TMZ re-sensitization or single-agent activity for recurrent GBM.

ACTR-20. A SMALL MOLECULE AXL INHIBITOR, BGB324 – FIRST-IN-HUMAN GBM SURGICAL PK TRIAL FOR RECURRENT TUMORS

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Glioblastoma (GBM) remains the deadliest of all primary brain tumors with very few effective treatment options. Recently, we reported that high AXL expression is correlated with poor prognosis in GBM patients and demonstrated the therapeutic benefits of targeting AXL, a member of TAM receptor tyrosine kinase family using a novel small molecule inhibitor, BGB324 in immunocompetent mouse GBM models and xenografts of patient-derived glioma stem cells(GSCs). The promise of BGB324 in tumor burden management prompted us to develop a clinical trial with BGB324 as a single agent therapeutic with the goal to extend it as a combinatorial therapy in the future. Our surgical PK/PD clinical trial with BGB324 in recurrent GBM has been approved by the Brain Malignancy Steering Committee at the National Cancer Institute. Study treatment will consist of 2 cohorts of adult GBM patients, one (Group A) receiving the treatment preoperatively and the other (Group B) receiving no treatment at all prior to surgery. First 5 patients recruited to Group A will be checked for the desired intra-tumoral drug concentration achieved to continue the trial. Group A will be supplemented by an additional 5 patients bringing the number to n=10 in each arm of the trial. Following surgical resection, patients in both cohorts will receive BGB324 daily in 21-day cycles. Treatment will be continued unless patients exhibit significant toxicity or substantial tumor progression. Our preclinical findings show the upregulation of AXL and its role in apoptosis induction in mesenchymal GBM as well as its association with MLK4, a serine threonine kinase we previously characterized as a mesenchymal GSC molecular target. Inhibition of phosphorylation of AXL and concomitant NF-kB activation in mesenchymal GSCs was found to be the nodal target of the drug action. An up-to-date information of the trial will be presented in detail.

ACTR-21. MANAGEMENT OF OCULAR SIDE EFFECTS IN PATIENTS WITH EGFR-AMPLIFIED GLIOBLASTOMA RECEIVING DEPATUXIZUMAB MAFODOTIN

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BACKGROUND: Depatuxizumab mafodotin (depatux-m, formerly ABT-414), is an antibody-drug conjugate comprised of an EGFR-targeted antibody, a non-cleavable linker maleimidocaproyl, and the microtubule inhibitor monomethylauristatin F. Promising antitumor activity of depatux-m was observed in patients with glioblastoma (GBM) in Phase 12 studies. Dosage of depatux-m is limited by ocular side effects (OSE), such as blurred vision, dry eye, and photophobia from corneal epitheliopathy, which are generally reversible after dose reduction or drug discontinuation. This Phase 3b study evaluates depatux-m-related OSE management strategies used in depatux-m clinical trials. METHODS: This open-label study will enroll approximately 90 patients with newly diagnosed, histologically confirmed, grade IV GBM that is epidermal growth factor receptor (EGFR)-amplified. Patients will receive depatux-m during the chemoradiation phase (radiation and temozolomide [TMZ]), and during adjuvant therapy with TMZ. Patients are randomized to one of three prophylactic ocular treatments: standard steroids (SS), SS with vasoconstrictors and cold compress (VC), or enhanced steroids (ES) with VC. Primary objective is to evaluate these prophylactic strategies for their effect on the proportion of patients requiring a change in OSE management due to inadequate control of OSEs, defined as either a 3-line decline in visual acuity from baseline or Grade 3 OSE severity on the Corneal Epithelial Adverse Event (CEAE) activities of daily living-based scale. Inadequate control with initial prophylactic regimen will trigger a switch to the addition of bandage contact lenses (BCL). Secondary objective assesses change in OSE management due to inadequate control of OSEs by BCL, defined as percentage of patients with Grade 3 CEAE that will trigger transition of patient to investigator discretion regimen (depatux-m interruption/dose reduction, VC prophylaxis, or ES prophylaxis). ClinicalTrials.gov: NCT03419403.

ACTR-22. A PHASE I STUDY OF CYTOSINE DEAMINASE-EXPRESSING NEURAL STEM CELLS (CD-NSCs) ADMINISTERED INTRACRANIALLY AND IN COMBINATION WITH ORAL 5-FLUOROCYTOSINE (5-FC) AND LEUCOVORIN IN PATIENTS WITH RECURRENT HIGH GRADE GLIOMA

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BACKGROUND: Human NSCs are tumor tropic, making them attractive vehicles for delivery of therapeutics. An immortalized, clonal NSC line was retrovirally transduced to express CD, which converts 5-FC to 5-fluorouracil (5-FU). The primary objectives of this study were to assess the feasibility of serially administering CD-NSCs intracranially via a Rickham catheter and determine the recommended doses for phase II testing (RP2D). METHODS: Adult patients with recurrent high grade gliomas underwent tumor resection or biopsy and placement of a Rickham. CD-NSCs were injected during surgery and thereafter infused through the Rickham every 2 weeks. Three days after each dose of CD-NSCs, patients took 5-FC (and leucovorin—dose level 3 patients only) orally every 6 hours for 7 days. The dose of CD-NSCs was escalated from 50 x 10^6 to 150 x 10^6 using a standard 3 + 3 design. 5-FC and leucovorin doses were 37.5 mg/kg and 25 mg, respectively. A treatment cycle was 28 days, with CD-NSCs administered on days 1 and 15, followed by 5-FC (and leucovorin) on days 4–10 and 18–24. Blood samples were drawn to assess for possible anti-NSC antibody and T cell responses. RESULTS: Fifteen evaluable patients received a median of 2 (range 1–5) cycles of study treatment. One dose-limiting toxicity occurred: grade 3 wound infection. Three patients developed anti-NSC antibodies after receiving 3 doses of NSCs. There was no correlation between these results and use of dexamethasone or number of cycles. Analyses of PK and possible anti-NSC T cell responses are ongoing. Three patients had stable disease for 5 months. CONCLUSIONS: Use of a Rickham to serially administer CD-NSCs intracranially is safe and feasible. Study treatment was well tolerated. There were no clinical signs of immunogenicity to these allogeneic CD-NSCs. The RP2D is 150 million CD-NSCs, 37.5 mg/kg of 5-FC, and 25 mg of leucovorin per dose.

ACTR-23. SAFETY OF INTRA-ARTERIAL CHEMOTHERAPY WITH OSMOTIC OPENING OF THE BLOOD-BRAIN BARRIER

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Intra-arterial (IA) infusion of hypertonic mannitol transiently opens the blood-brain barrier (BBB) to improve drug delivery to intracerebral tumors. The aim of this study was to evaluate the safety of osmotic BBB disruption (BBBD) followed by administration of IA chemotherapy. We performed a retrospective chart review of all malignant brain tumor patients who underwent BBBD on six IRB approved treatment protocols or off protocol at Oregon Health and Science University between 1997 and 2017. Toxicities and adverse events (AEs), including death within 30 days of treatment, were assessed. A total of 4018 BBBD procedures were performed on 268 patients (mean 15 BBBD procedures per patient). The most common pathologies were primary central nervous system lymphoma (32%) and anaplastic oligodendroglioma (12%). Most AEs were chemotherapy-related. Only 5% of AEs were attributable to the BBBD procedure, and only 0.42% of these were associated with permanent neurological damage (Grade 3 or 4 SAE). Four SAEs were due to ischemia as detected on magnetic resonance imaging and had minimal impact on quality of life. Four SAEs were due to anterior cord syndrome subsequent to iatrogenic laminar flow of the chemotherapy and were partially responsive to steroids. Subsequently this toxicity was eliminated by procedures to avoid laminar flow. Focal seizures, largely responsive to medical intervention, occurred within 24 hours after 257 (6.4%) BBBD procedures. Most seizures (229, 89%) followed IA administration of methotrexate and were transient and without sequelae. Five patient deaths occurred within 30 days; 1 due to a brain stem stroke related to BBBD, 1 due to a pulmonary embolus, and 3 due to disease progression. We conclude that although the BBBD procedure is invasive, permanent toxicities or death are rare. These results show that osmotic BBBD can be performed safely in brain tumor patients.

ACTR-25. UPDATED RESULTS FROM A PROSPECTIVE, RANDOMIZED PHASE 2 STUDY IN PATIENTS WITH FIRST RELAPSE OF HIGH-GRADE ASTROCYTOMA USING TVB-2640 IN COMBINATION WITH AVASTIN VERSUS AVASTIN ALONE

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BACKGROUND: Standard of care for glioblastoma multiforme (GBM) is surgical resection followed by temozolamide, with Avastin given at relapse. Responses to Avastin remain brief; resistance may involve overexpression of Fatty Acid Synthase (FASN). Our institution is conducting a phase 2 study of Avastin with or without the FASN inhibitor TVB-2640 in patients with GBM in first relapse. METHODS: This is a prospective, randomized, phase 2 study of Avastin with or without TVB-2640 in patients with GBM in first relapse. Primary end point is progression free survival (PFS). Inclusion criteria are: age 18, ECOG 0 to 2, GBM progression following standard combined modality treatment. Randomization is into 2 separate arms. Patients in arm 1 receive Avastin every 2 weeks in combination with TVB-2640. Patients in arm 2 receive Avastin alone every 2 weeks. MR-Spectroscopy (MRS) and serum sampling for exosome analysis will be obtained on all patients at day 1 and 28 of first cycle. Starting on cycle 2 day 1, all patients will converge to a single arm and will continue to receive Avastin in combination with TVB-2640. A total sample size of 24 patients will provide 90% power to detect a 4 month difference in PFS (3 months for Bev alone (historic controls) versus 7 months for TVB-2640 in combination with Bev, (i.e., a hazard ratio of 0.43) using a one-sided log-rank test with alpha=0.1. RESULTS: We have enrolled 13 patients to date, 12 have started therapy; 1 came off study early due to intracranial hemorrhage. No grade 3 or higher treatment related AEs have occurred. Updated results will include PFS, response, and biomarker analysis (exosome, MRS). CONCLUSIONS: The combination of TVB2640 with Avastin appears to be well tolerated. Enrollment will continue with planned completion in early 2019. (Clinical trial registry number: NCT03032484).

ACTR-26. A FEASIBILITY STUDY OF THE NATIVIS VOYAGER® SYSTEM IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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BACKGROUND: The Nativis Voyager® system is a non-sterile, non-invasive, non-thermal, portable, investigational medical device that uses a specific, localized ultra-low radio frequency energy (ulRFE®) cognate for the treatment of brain cancer. METHODS: In this prospective, open-label, multi-center trial, adults newly diagnosed with GBM, following maximal tumor debulking, are eligible for enrollment. The objective of the study is to assess if the Voyager ulRFE therapy is a safe and feasible treatment for newly diagnosed GBM when combined with standard of care (i.e., focal radiotherapy + temozolomide). Patients receive continual therapy with the Voyager, concurrently with radiotherapy + temozolo-

mide. Upon progression, investigators can choose to maintain patients on study with the Voyager and to add second-line therapy. The primary outcome measure is safety, which is assessed by the incidence and evaluation of any adverse events associated with the Voyager through follow-up. The secondary outcome measure is clinical utility, which is assessed by progression-free survival and overall survival. RESULTS: Eleven patients were enrolled at 3 centers into the first (safety) cohort of this study. No device-related adverse events were reported by patients receiving concurrent therapy with Voyager + radiotherapy + temozolomide. All patients remain progression-free and on study as of June 2018. Given the safety profile, enrollment was expanded to 32 patients. Additional interim safety and clinical utility data will be reported. CONCLUSIONS: The Nativis Voyager system appears to be safe and feasible for the treatment of GBM. Given that therapy is delivered non-invasively and no device-related adverse events were reported, further prospective study of the investigational device is warranted.

ACTR-27. PHASE 2 STUDY OF DIANHYDROGALACTITOL (VAL-083) IN PATIENTS WITH MGMT-UNMETHYLATED, BEVACIZUMAB-NAÏVE RECURRENT GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive primary brain cancer. Current standard-of-care includes surgery followed by concurrent therapy with radiation and temozolomide (TMZ) and maintenance TMZ. Almost all GBM patients experience recurrent/progressive disease, and median survival after recurrence is 3–9 months. Effective therapies for recurrent GBM (rGBM) are lacking, representing a significant unmet medical need. Unmethylated promoter for O6-methylguanine-DNA-methyltransferase (MGMT) is a validated biomarker for TMZ-resistance and is correlated with a poor prognosis. Second-line treatment with the anti-angiogenic agent bevacizumab (BEV) has not improved survival, and 5-year survival is less than 3%. VAL-083 is a bi-functional DNA-targeting agent rapidly inducing interstrand cross-links at N7-guanine, leading to DNA double-strand breaks and cell-death. VAL-083s cytotoxicity is independent of MGMT status, and VAL-083 overcomes TMZ-resistance in GBM cell lines, GBM cancer stem cells, and in vivo GBM models. We completed a 3 + 3 dose-escalation trial of VAL-083 in TMZ- and BEV-refractory rGBM. 40mg/m2/day given on days 1,2,3 of a 21-day cycle was generally well-tolerated, and this dose was selected for further clinical evaluation in Phase 2 trials. The trial described here is an ongoing single-arm, biomarker-driven Phase 2 trial in MGMT-unmethylated BEV-naïve adult rGBM. In this trial, 48 patients will receive VAL-083 40mg/m2/day on days 1,2,3 of a 21-day cycle. Tumor response will be assessed by MRI approximately every 42 days, per RANO criteria. The primary objective of this study is to determine if VAL-083 improves median overall survival (mOS) for MGMT-unmethylated rGBM patients compared to a historical mOS of 7.1 months for such patients treated with lomustine (EORTC26101). Secondary efficacy endpoints include progression-free survival (PFS), overall response rate (ORR), duration of response (DOR), and quality-of-life (QOL) evaluation using the MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) self-reporting tool. Enrollment and safety data update will be provided at the meeting. Clinicaltrials.gov identifier: NCT02717962.

ACTR-28. PHASE 1 DOSE ESCALATION TRIAL OF THE SAFETY OF BMX-001 CONCURRENT WITH RADIATION THERAPY AND TEMOZOLOMIDE IN NEWLY DIAGNOSED PATIENTS WITH HIGH-GRADE GLIOMAS

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BACKGROUND: BMX-001 (MnTnBuOE-2-PyP5+) is a metalloporphyrin with differential action in response to oxidative stress caused by radiation therapy (RT)and chemotherapy, both critical components in the treatment of high-grade gliomas (HGG). In preclinical studies, BMX-001 functions as a radioprotectant of normal tissue, for example protection of central nervous system white matter and radiosensitizer of human glioblastoma (GBM) xenografts. Therefore, we underwent a phase 1 study to evaluate safety of BMX-001 in newly diagnosed patients with HGG receiving concurrent RT and temozolomide (TMZ). METHODS: We performed a phase 1, single-center, dose-escalation study of BMX-001 in combination with concurrent RT (daily fractions of 1.8–2 Gy given 5 days/week for 6 weeks for a total of 59.4–60 Gy) and TMZ (75 mg/m2 daily X 42 days). We administered BMX-001 as a subcutaneous injection at a loading dose before start of RT and TMZ and then subsequently 2 times/week for 8 weeks. Primary endpoint was determination of the maximum tolerated dose (MTD). We assessed safety using National Cancer Institute Common Terminology Criteria for Adverse Events 4.03. RESULTS: We enrolled 15 subjects with GBM (WHO grade IV) with age range of 19 to 80 years. BMX-001 at 42 mg loading dose and 20 mg subsequent dose was the maximum administered dose and 28 mg loading dose and 14 mg subsequent dose was the MTD. Sinus tachycardia (grade 3) was the dose-limiting toxicity at 42 mg loading dose (n=1). Only other related grade ≥ 3 event seen was hypotension (grade 3) (n=1). Most common related toxicity was grade 1 injection site reaction (n=7). CONCLUSIONS: BMX-001 with RT plus TMZ and post-RT TMZ for patients with newly diagnosed HGG was safe and well-tolerated. Phase II study with BMX-001 in combination with concurrent RT and TMZ in subjects with newly diagnosed HGG is planned.

ACTR-29. KETOGENIC DIETS AS AN ADJUVANT THERAPY IN GLIOBLASTOMA (KEATING): A MIXED METHOD APPROACH TO ASSESSING TRIAL FEASIBILITY.

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BACKGROUND: There is increasing interest in the use of ketogenic diets (KD) as adjuvant therapy for glioblastoma (GBM). This trial aimed to: (i) investigate protocol feasibility, (ii) measure patient/carer acceptability and (iii) inform phase III trial design. METHODS: A prospective, randomised, feasibility study, with an embedded qualitative design. Twelve newly diagnosed GBM patients were randomised to the modified ketogenic diet (MKD) or the medium chain triglyceride ketogenic diet (MCTKD). Primary outcome was retention; secondary outcomes included recruitment rate, side effects, extent of ketosis and dietary acceptability, assessed at 12 weeks and 12 months. Semi-structured interviews were conducted with a representative sample of patients and relatives (n=15). RESULTS: Recruitment was achieved within the defined timeframe of 12 months; 42 patients were eligible, of which 12 were recruited (29% recruitment rate). Retention was poor; of the 12 patients randomised, 4 completed the 12-week dietary trial (3 on MCTKD; 1 on MKD). Median duration on diet was 36.5 days (range 0–49) for those who discontinued prior to 12 weeks (n=8). Those who completed the 12-week intervention (n=4) achieved ketosis (1.65mmol/L 1.3mmol/L). No dietary related serious adverse events occurred. Qualitative interviews revealed patients who completed the 12-week diet consented without hesitation. Those who declined participation pre-empted the issues experienced by those who withdrew prior to 12-weeks; namely high levels relative/carer burden and social exclusion. Retainers had a positive experience and reported the diet became the new normal. Patients stated that the 3-month diet duration was too long, but 6 weeks would be more attainable. CONCLUSION: Recruitment to a GBM ketogenic trial is feasible. Retention at 3 months was poor due to the diet negatively impacting patient lifestyle and causing burden for relatives. To assess efficacy in a phase III trial, a 6-week dietary intervention would be advantageous.

ACTR-30. PHASE 1B/2 STUDY TO ASSESS THE CLINICAL EFFECTS OF PAMIPARIB (BGB-290) IN COMBINATION WITH RADIATION THERAPY (RT) AND/OR TEMOZOLOMIDE (TMZ) IN PATIENTS WITH NEWLY DIAGNOSED OR RECURRENT/REFRACTORY GLIOBLASTOMA (GBM)

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DNA damage caused by TMZ or RT sensitizes tumors to PARP inhibitors, especially in highly replicating tumors (eg, GBM). Pamiparib is a selective PARP1/2 inhibitor with potent PARP trapping that can cross the blood-brain barrier and has shown synergistic cytotoxicity with TMZ in non-clinical experiments. At 60mg BID, the human-equivalent dose-to-trough brain concentrations above the nonclinical efficacy threshold, pamiparib was generally well tolerated and showed antitumor activity in early clinical studies (NCT02361723; NCT03333915). This ongoing dose-escalation/expansion study (NCT03150862) will determine the safety/tolerability and antitumor effects of pamiparib (60mg BID)+RT and/or TMZ. The dose-escalation component consists of three arms. Arm A will establish tolerable duration of pamiparib (2, 4, 6 weeks)+RT in newly diagnosed GBM patients with unmethylated MGMT promoter (unmethyl-GBM). In Arm B, newly diagnosed patients with unmethyl-GBM will receive pamiparib+RT with increasing TMZ doses. Enrollment in Arm B will commence once RP2D for pamiparib+RT is established. In Arm C, patients with recurrent/refractory methylated- or unmethyl-GBM receive pamiparib with increasing TMZ doses. As of 28 March 2018, 15 patients were enrolled (A: 2-wk, n=3; 4-wk, n=6; C: TMZ [40mg], n=6). One DLT (grade 3 nausea) was reported in Arm C. Across arms, pamiparib-related AEs occurring in >3 patients were nausea (n=6) and fatigue (n=5). Two patients experienced three pamiparib-related AEs grade 3 (diarrhea [A: 4-wk, n=1]; fatigue and nausea [C: n=1]). All three resolved with concomitant medication and treatment interruption (A) or discontinuation (C). Of the seven patients with 1 tumor assessment, one (A: 4-wk) achieved an unconfirmed PR; four (A: 2-wk, n=2; 4-wk, n=2) had SD, and two (A: 2-wk, n=1; C: n=1) had PD. Preliminary data suggests pamiparib at 60mg BID is generally well tolerated by patients when administered 4 weeks concurrently with RT for newly diagnosed unmethyl-GBM and when combined with 40 mg TMZ for recurrent/refractory GBM.

ACTR-31. PHASE 1 STUDY OF AG-881, AN INHIBITOR OF MUTANT IDH1 AND IDH2: RESULTS FROM THE RECURRENT/PROGRESSIVE GLIOMA POPULATION

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INTRODUCTION: Isocitrate dehydrogenase 1 and 2 mutations (mIDH1/2) occur in >70% of low-grade gliomas and secondary glioblastomas, and lead to genetic and epigenetic dysregulation, promoting tumorigenesis. AG-881 is an oral, potent, brain-penetrant inhibitor of mIDH1/2 under phase 1 clinical evaluation in gliomas and other solid tumors. Here we present clinical data from the glioma population. METHODS: Patients with recurrent/progressive mIDH1/2 glioma received AG-881 daily in continuous 28-day cycles. A Bayesian model was used for dose escalation. Dose-limiting toxicity (DLT) definition: Grade 3 AG-881-related adverse event (AE) in Cycle 1 or by sponsor designation. Blood samples were collected for pharmacokinetic (PK)/pharmacodynamic (PD) evaluations. MRI response every 8 weeks by RANO and RANO-LGG criteria. RESULTS: As of 28Mar2018, 52 patients with glioma had received AG-881 and 17 (32.7%) remained on treatment. Grade 2/3 = 90.4%; median age = 42.5 years; IDH1/2: 48/3; median no. prior therapies = 2 (range 1–6). Five initial dose levels tested: 25mg (n=6), 50mg (n=5), 100mg (n=10), 200mg (n=14), and 300mg (n=5). To confirm safety and PK, a 10mg dose level was tested (n=6) and 6 additional patients enrolled in the 50mg cohort. Common (>20%) AEs across glioma patients regardless of attribution: ALT increased (44.2%), AST increased (38.5%), headache (34.6%), fatigue (30.8%), nausea (26.9%), seizure (21.2%). Five patients experienced DLTs at 100mg: Grade 2 ALT/AST that resolved to Grade 1 with dose modification (n=4) or discontinuation (n=1). Among the evaluable glioma population: 2% minor response, 75% stable disease, 21% progressive disease, and 2% missing as best overall response. CONCLUSION: Maximum tolerated dose/recommended phase 2 dose was not reached by Bayesian model; clinical team recommendation was to proceed with doses <100mg in patients with glioma. The 10mg and 50mg doses are being explored in an ongoing perioperative glioma study. Updated safety, PK, and imaging response analyses will be presented.

ACTR-32. 5-ALA FLUORESCENCE IS A POWERFUL MARKER FOR DETECTION OF UNEXPECTED GLIOBLASTOMA TISSUE DURING SURGERY OF RADIOLOGICALLY SUSPECTED LOW-GRADE GLIOMAS

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BACKGROUND: Precise tissue sampling during resection of suspected low-grade gliomas (LGG) is the basis for an accurate histopathological diagnosis to enable adequate patient management. In the course of malignant transformation of initial LGG, small intratumoral areas of glioblastoma tissue can potentially arise that might be unrecognized during surgery and thus result in treatment failure. Recently, 5-aminolevulinic acid (5-ALA) induced fluorescence was identified as intraoperative marker for visualization of focal intratumoral WHO grade III areas. The aim of this study is thus to clarify if 5-ALA is also capable to identify areas of unexpected glioblastoma tissue during surgery of radiologically suspected LGG. METHODS: Our database at the Medical University of Vienna and University of California, San Francisco was screened for adult patients with 5-ALA fluorescence-guided resection of a suspected glioma with non-significant MRI contrast-enhancement (CE; no, patchy/faint or focal CE). In this study, only patients with newly diagnosed lesions were included. In contrast, recurrent gliomas and biopsy only cases were excluded. In all patients, histopathological diagnosis was established according to the WHO classification. RESULTS: Altogether, 7 patients (median age: 53 years, range: 30–66 years) with histological diagnosis of a glioblastoma were identified despite initial radiological suspicion of LGG. Of these, no CE was found on preoperative MRI in two cases (29%), patchy/faint CE in two cases (29%) and focal CE in three cases (42%). During surgery, intratumoral areas with focal 5-ALA induced fluorescence were observed in all 7 patients. In contrast, no visible fluorescence was found in the remaining intratumoral regions. CONCLUSIONS: Our study indicates that 5-ALA induced fluorescence is able to identify intratumoral areas containing even focal glioblastoma tissue in radiologically suspected LGG. Thus, the 5-ALA technique will in future markedly improve tissue sampling during resection of suspected LGG to allow a precise histopathological diagnosis and optimized postoperative patient management.

ACTR-33. TUMOR TISSUE PENETRATION AND PHARMACODYNAMICS OF ONC201 IN ADULT RECURRENT GLIOBLASTOMA PATIENTS

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BACKGROUND: ONC201 selectively antagonizes DRD2, crosses the blood-brain barrier and induces apoptosis in high grade gliomas and other advanced cancers. ONC201 efficacy is pronounced in glioma cells that harbor low DRD5 expression and is associated with induction of the ATF4/CHOP/DR5-mediated integrated stress response pathway. DRD2 antagonism also induces activation of NK and other immune cells. We previously reported the single agent activity of ONC201 in 17 adult patients with recurrent glioblastoma that demonstrated the safety, systemic pharmacodynamics, and a durable objective response when administered orally once every 3 weeks. Here, we evaluated the intratumoral drug concentrations and pharmacodynamic activity of ONC201 in adult recurrent glioblastoma patients that were treated on a weekly schedule. METHODS: Six patients >18 years old with first recurrence of glioblastoma who were eligible for salvage surgical resection were enrolled. ONC201 was administered orally as 625 mg once a week. Salvage surgery was performed approximately 24 hours after the second dose of ONC201 and patients continued on ONC201 until radiographic and/or clinical progression. Tumor tissue was flash-frozen and formalin-fixed for assessment of intratumoral drug concentrations by

LC-MS and pharmacodynamics by IHC, respectively. RESULTS: Of the 6 enrolled patients, 5 had sufficient tissue for evaluation. Intratumoral drug concentrations exceeded therapeutic thresholds: median 1.5 μM (range 600 nM 9.3 μM). Investigation of biomarkers related to downstream signaling induced by ONC201 revealed heterogeneous intratumoral induction of ATF4/CHOP/DR5 expression and tumor cell apoptosis (TUNEL). Stronger induction of the pharmacodynamic signaling was associated with stronger induction of apoptosis and inversely associated with DRD5 expression, but not with drug concentration. No drug-related adverse events were reported in this cohort. Evaluation of immune cytokines and effectors molecules in serum and immune infiltration into the tumor is ongoing. CONCLUSIONS: ONC201 is biologically active and well tolerated in recurrent glioblastoma tumors when administered to adults.

ACTR-34. INTEGRATED CLINICAL EXPERIENCE WITH ONC201 IN PREVIOUSLY-TREATED H3 K27M-MUTANT GLIOMA PATIENTS

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BACKGROUND: H3 K27M-mutant gliomas have a dismal clinical prognosis and no proven curative therapy. We report the updated clinical experience with ONC201, the first cancer-specific DRD2 antagonist, in adult and pediatric H3 K27M-mutant gliomas. METHODS: As of May 28, 2018, 26 patients with H3 K27M-mutant glioma have been treated with ONC201: 9 pediatric (<18 years old) and 17 adult patients (>18 years old). Twelve patients had recurrent disease and 14 had previously-treated stable disease prior to initiating ONC201. Patients had 1–4 prior lines of therapy and all received prior radiation. Ten adult patients were enrolled on clinical trials and the other 16 were on compassionate use. ONC201 was orally administered at 625 mg to adults and scaled by body weight for pediatric patients. All patients, except one, were dosed weekly. RESULTS: Fourteen of 26 patients (54%) remain progression-free on ONC201 with a median follow up of 3.6 (range 1.6–24.5) months. No dose modifications or discontinuation due to toxicity have occurred. Among the 12 adult patients with recurrent disease who received single agent ONC201, the estimated PFS6 is 36.5%. Seven patients have experienced radiographic and/or clinical benefit (neurological stabilization or improvement). Among the 5 adults with recurrent thalamic glioma, three have experienced durable complete regression of their thalamic tumors, including the first H3 K27M-mutant glioma patient treated with ONC201 who has experienced 96% regression of her recurrent disease and continues single agent ONC201 for >2 years. Two DIPG pediatric patients who initiated single agent ONC201 6–8 weeks after radiation have experienced radiographic and neurological improvements and exhibited PFS of >13 months from diagnosis. CONCLUSIONS: Emerging clinical data suggest that ONC201 exhibits clinical activity for some patients with H3 K27M-mutant glioma.

ACTR-35. MOLECULAR MATCHMAKING: EFFICACY OF EARLY CLINICAL TRIALS GUIDED BY NEXT-GENERATION SEQUENCING IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

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INTRODUCTION: Next-generation sequencing (NGS) is available for analysis of tumors in patients with glioblastoma multiforme (GBM) and can be used to match patients to clinical trials of agents that target specific alterations. We studied the relationship between molecular matching by NGS and treatment outcome in GBM patients treated on trials of targeted agents. METHODS: Patients were adults with GBM who enrolled on clinical trials at our center. Patients underwent NGS with either FoundationOne (tissue) or Guardant360 (cell-free DNA) prior to enrollment on a trial. Treatment was classified as a direct match, indirect match, or non-match. Clinical benefit was defined as complete response, partial response, or at least 4 months on treatment without progressive disease by RANO criteria. RESULTS: 19 consecutive patients underwent NGS and were treated on 1 or more of 8 different clinical trials, including 4 patients who were treated on 2 trials, for a total of 23 cases analyzed. 10 patients were male; 9 were female. Median age was 58 years (range 26–73). Median number of prior systemic therapies was 1. Three cases were a direct match, 8 cases were an indirect match, and 12 cases were a non-match. Clinical benefit was observed in 54.5% of patients with a direct or indirect match vs. 8.3% of patients with a non-match. Mean treatment duration was 252 days for patients with a direct or indirect match vs. 57 days for patients with a non-match. CONCLUSION: GBM patients treated on clinical trials of targeted agents that were a direct match or indirect match had a trend of longer duration of treatment and higher rate of clinical benefit, compared to trials that were a non-match. These observations support the use of next-generation sequencing to identify clinical trials for patients with GBM.

ACTR-36. A SINGLE ARM PHASE 2 STUDY OF THE DUAL mTORC1/mTORC2 INHIBITOR VISTUSERTIB PROVIDED ON AN INTERMITTENT SCHEDULE FOR NEUROFIBROMATOSIS 2 PATIENTS WITH PROGRESSIVE OR SYMPTOMATIC MENINGIOMAS

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Meningiomas are the second most common tumor in NF2 patients, with a cumulative prevalence of 80% by age 70 and a high prevalence of multiple meningiomas. Surgery remains standard of care for these tumors, yet outcomes remain suboptimal for many patients with multiple tumors. Loss of NF2 expression is associated with activation of the mTOR pathway, but treatment with the mTORC1 inhibitor everolimus is not associated with tumor shrinkage. We hypothesized that inhibition of both mTORC1 and mTORC2 pathways would result in increased activity against meningiomas. This single center, phase II, open label study evaluated adults (18 years) with NF2 and progressive meningiomas treated with vistusertib. Subjects received vistusertib 125 mg BID two consecutive days per week. Radiographic response was defined as 20% decrease in tumor volume from baseline. The primary endpoint was radiographic response rate in the target meningioma; secondary endpoints included radiographic response in non-target meningiomas and vestibular schwannomas. We enrolled 18 subjects (5M;13F) with a median age of 40 years (range 18–60 years). Baseline volume of target meningioma was 14.2 ml (range, 3.3–69.2 ml) and median pretreatment growth rate was 33%/year. A radiographic response was seen in 1/18 (6%) target meningiomas and in 2/20 (10%) of non-target meningiomas and in 2/21 (9.5%) of vestibular schwannomas. Three target meningiomas (17%) progressed during treatment. Seven subjects (39%) discontinued treatment by choice due to tolerability and 8 remain on study. Adverse events included fatigue, nausea, vomiting, anorexia, rash, mucositis, and hypophosphatemia. Vistusertib treatment is associated with radiographic response rates of 5–10% in meningiomas and schwannomas in NF2 patients, with only a minority of meningiomas progressing during treatment. Grade 2 toxicity led to treatment discontinuation in a significant minority of patients. Future analyses will address the relationship between tumor shrinkage and activation of TORC1/2 pathways in archival tumor specimens.

ACTR-37. SHORT-TERM BEVACIZUMAB FOR RECURRENT GLIOBLASTOMAS

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Survival benefit of bevacizumab (BEV) could not be confirmed for recurrent glioblastomas in EORTC 26101, while BEV is commonly chosen at recurrence due to its positive effect in daily life. BEV is usually repeated until tumor progression or adverse events, but the benefit of continuation of BEV is not known. Recently, short-term BEV has been administrated in our institute to avoid from adverse events, and to reduce medical costs. The survival data of the patients treated with short-term BEV are retrospectively analyzed. Thirty-two patients, who presented recurrence of primary glioblastoma, received short-term BEV, which is 10 mg/kg of BEV bi-weekly administration for 2–6 courses. BEV was discontinued after favorable response in MRI and social functioning, and was resumed for further progression. When the tumor progressed after short-term BEV, it was continued to delay neurological deterioration. Twenty-three of 32 patients could discontinue BEV in the first administration, and 13 of 23 patients were treated with second short-term BEV for further progression. Six of 13 could discontinue BEV again. In our retrospective cohort, median post-recurrent survival was 8.0 months, and 9- and 12-months survivals are 39.8% and 31.8%. The patients, who were able to discontinue BEV, showed statistically longer

survival than the patients, who were not, 11.0 months and 3.5 months (p=0.0002, Log-rank). BEV could be safely discontinued for the patients, who respond well for the first administration, and the initial response to BEV might be a good prognostic factor at recurrence.

ACTR-38. A PHASE I TRIAL OF AFATINIB AND RADIOTHERAPY (RT) WITH OR WITHOUT TEMOZOLOMIDE (TMZ) IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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GBM is the most frequent primary CNS tumor. RT + TMZ represents first-line therapy. ErbB pathway dysregulation contributes to GBM pathogenesis; EGFR activation is associated with RT resistance. This 3 + 3 dose-escalation study assessed afatinib, an irreversible ErbB family blocker, with RT ± TMZ in newly diagnosed GBM. Patients with MGMT promoter methylation received afatinib (20, 30, 40 mg/day) + RT + TMZ for 6 weeks (RT period), then afatinib 40 mg/day + TMZ for 6 months, then afatinib 40 mg/day until progression/undue adverse events (AEs; Regimen M). Those with unmethylated MGMT promoter received RT + afatinib then afatinib (Regimen U). Primary endpoint was maximum tolerated dose (MTD) of afatinib + RT ± TMZ; secondary endpoints were safety, pharmacokinetics and antitumor activity. Thirty-six patients were enrolled (M, 20; U, 16). In regimen M, 1/6 (20 mg), 0/6 (30 mg) and 2/5 (40 mg) evaluable patients had dose-limiting toxicities (DLTs) in the RT period (two Grade 4 thrombocytopenia, one Grade 3 vomiting); MTD of afatinib + RT + TMZ was 30 mg/day. In regimen U, 0/3 (20 mg) and 1/6 (40 mg) evaluable patients had DLTs (Grade 3 diarrhea); MTD of afatinib + RT was 40 mg/day. Common treatment-related AEs were diarrhea, rash, fatigue, nausea and thrombocytopenia; 80% and 75% had Grade 3 AEs in M and U. Pharmacokinetic evaluation suggested that afatinib with RT ± TMZ had no influence on afatinib exposure. Five patients in M and one in U had an objective response. Five patients (M, 4; U, 1) were long-term responders to afatinib (>12 months treatment); two had available tumor samples. Both had MGMT promoter methylation; one had a PTPN11 mutation, the other focal EGFR amplification with concomitant EGFRvIII allele amplification. Afatinib + RT ± TMZ appears tolerable; preliminary biomarker analysis may indicate patients likely to have long-term responses.

ACTR-39. TWO-YEAR RESULTS OF THE INTELLANCE 2/EORTC TRIAL 1410 RANDOMIZED PHASE II STUDY ON DEPATUX–M ALONE, DEPATUX-M COMBINED WITH TEMOZOLOMIDE (TMZ) AND EITHER TMZ OR LOMUSTINE IN RECURRENT EGFR AMPLIFIED GLIOBLASTOMA (NCT02343406

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BACKGROUND: Depatux-M is an antibody-drug-conjugate consisting of an antibody (ABT-806) specific to the activated conformation of EGFR bound to the toxin monomethylauristatin-F. In the primary analysis on EORTC 1410 we reported a trend (p = 0.06) towards improved overall survival (OS) in patients with EGFR-amplified (amp) recurrent glioblastoma treated with Depatux-M in combination with temozolomide. METHODS: Eligible were patients with centrally confirmed EGFRamp glioblastoma at 1st recurrence after temozolomide chemo-irradiation. Patients were randomized to either a) Depatux-M 1.0 mg/kg every 2 weeks intravenously, or b) the same treatment combined with temozolomide 150–200 mg/m2 day 1–5 every 4 weeks, or c) either lomustine or temozolomide (TMZ/LOM) depending on the time of relapse. Primary endpoint was OS. Pharmacokinetic sampling was part of the study design, all samples were used to calculate the Depatux-M average concentration during course 1 (CavgC1). The level of EGFRamp was re-analysed using next generation sequencing. RESULTS: In February 2018, an updated OS comparison performed after 220 observed deaths of Depatux-M in combination with TMZ versus TMZ/LOM using log-rank test and cox models stratified by stratification factors at randomization showed a HR of 0.68 (95%CI [0.48, 0.95]; p = 0.024) and 1-year OS rates of 40% versus 28%. In multivariate analysis CavgC1 was a significant predictor for OS (HR 0.96, 95% CI [0.93, 0.98], p = 0.0013). In Depatux-M treated patients, EGFR status (high vs low level amplification) did not correlate with OS. At the meeting the follow-up from Aug 2018 will be presented, obtained more than 24 months after the end of accrual. CONCLUSION: This updated OS analysis of Depatux-M in combination with temozolomide confirmed the OS improvement in EGFRamp recurrent glioblastoma. In Depatux-M treated patients, higher drug levels during course 1 were associated with improved OS, but high levels of EGFR amplification at first diagnosis were not.

ACTR-40. A PHASE 1, MULTICENTER, OPEN-LABEL STUDY OF MARIZOMIB (MRZ) WITH TEMOZOLOMIDE (TMZ) AND RADIOTHERAPY (RT) IN NEWLY DIAGNOSED WHO GRADE IV MALIGNANT GLIOMA (GLIOBLASTOMA, ndGBM): FULL ENROLLMENT RESULTS

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Proteasome inhibition sensitizes glioma cells to TMZ and RT, providing a novel therapeutic strategy for ndGBM. MRZ, an irreversible, brain-penetrant, pan-proteasome inhibitor with anti-glioma activity was combined with standard-of-care (SOC) concomitant TMZ/RT followed by adjuvant TMZ in ndGBM (NCT02903069), to determine the recommended dose (RD). Patients were enrolled in separate concomitant (TMZ/RT+MRZ, N=15) and adjuvant (TMZ+MRZ, N=18) cohorts in dose-escalation (3 + 3 design), followed by dose-expansion (N=20) at RD (0.8 mg/m2) in concomitant followed by adjuvant treatment. MRZ infused IV (10 min) at increasing dose levels (0.55, 0.7, 0.8, and 1.0 mg/m2): Concomitant days 1, 8, 15, 29, 36; Adjuvant days 1, 8, 15 (28-day cycle). RESULTS (as of 02May2018): Mean age 55 years, 68% male. Most common treatment-emergent adverse events (TEAEs, 20% patients, all grades): fatigue, nausea, vomiting, hallucination, ataxia, headache. Dose-limiting toxicities (DLTs): 1 (fatigue) at 0.7 mg/m2 adjuvant cohort, 3 (ataxia/diarrhea; ataxia/confusion; myocardial infarction) in concomitant and 2 (delirium/ataxia; ataxia/fatigue) in adjuvant cohorts at 1.0 mg/m2. Grade 3 TEAEs in 11 of 12 patients at 1.0 mg/m2 including one Grade 4 and one Grade 5 TEAE; at 0.8 mg/m2 MRZ, Grade 3 TEAEs in 9 of 21 patients. MRZ demonstrated a steep dose-response with TEAEs/DLTs predominately CNS AEs (ataxia, hallucinations) which were dose-related, short-lasting, reversible and ameliorated by early dose reductions, allowing patients to remain on treatment. Currently 8 dose-escalation patients remain active in Cycle 10–23. Median OS for dose-expansion not yet estimated; 7 patients remain active, 1 death, median follow-up 4.1 months. MRZ at the RD with adjuvant TMZ+Tumor Treating Fields (Optune) is currently enrolling. An international Phase 3 trial (EORTC #1709-BTG, NCT03345095) has been launched in 2018 to assess the overall survival benefit of MRZ added to SOC in ndGBM.

ACTR-42. THE USE OF ADVANCED DIFFUSION MRI PARAMETERS IN THE ASSESSMENT OF TREATMENT RESPONSE IN GLIOBLASTOMA USING MULTI-B VALUE ACQUISITION AND A HISTOGRAM-BASED APPROACH

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PURPOSE: Imaging biomarkers to assess early treatment response in gliomas are important for guiding optimal clinical decision-making for individual patients, and as platforms for multi-institutional clinical trials of novel therapies. Current Response Assessment in Neuro-Oncology (RANO) criteria, rely on subjective, semi quantitative measurements with limited sensitivity and specificity in the early treatment phase. Previous diffusion imaging studies have indicated early sensitivity to treatment response, however optimum acquisition and analysis approaches have yet to be established. The purpose of this study is to assess the utility of advanced quantitative diffusion MRI derived from multi B-value acquisitions in the assessment of treatment response, using a spatially independent approach. METHODS: We used least-square fitting to model six diffusion parameters from mono-, bi-, and stretch-exponential models, and performed a histogram analysis of voxels located within ROIs defined by enhancing tumour and increased signal on FLAIR sequences. Histograms were generated for 10 patients with GBM at 3 time-points before, at 6 weeks, and 3 months treatment with standard-of-care regimen (RT with concomitant and adjuvant temozolomide). Changes in the histograms percentile profiles were evaluated across the various time-points, back-projected onto MRI images for spatial correspondence, and compared with RANO assessment from structural MRI at the latest timepoint. RESULTS, DISCUSSION & CONCLUSION: Percentile profiles changed over the course of therapy, reflecting known biological changes in the tumour microenvironment in response to radiotherapy and chemotherapy; these preliminary data suggest differential changes in diffusion parameters early in treatment. Spatially-independent diffusion parameter comparisons abrogate the confound of voxel misregistration due to tumour growth/shrinkage. Further assessment of this dataset, augmented by on-going patient recruitment across multiple centres, will provide insight to the prognostic value of advanced diffusion MRI as a standardized method for treatment response assessment.

ACTR-43. OPEN-LABEL PHASE 1 CLINICAL TRIAL TESTING PERSONALIZED AND TARGETED SKULL REMODELING SURGERY TO MAXIMIZE TTFIELDS INTENSITY FOR RECURRENT GLIOBLASTOMA – INTERIM ANALYSIS AND SAFETY ASSESSMENT (OPTIMALTTF-1)

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BACKGROUND: We present a pre-specified interim analysis of an ongoing open-label, phase-1 IST (NCT02893137) testing safety/efficacy of a new rGBM treatment. The intervention combines personalized skull-remodeling (SR) surgery with TTFields and best-choice chemotherapy. SR-surgery involves minor craniectomy or burr-holes personalized to enhance TTFields intensity focally in the tumor. METHODS: Accrual began Dec 2016 (planned total 15 patients). Eligibility: Age > 18 years, first recurrence focal supratentorial GBM (RANO), and KPS 70. Patients were excluded upon progression, death, SUSARs, or unacceptable AEs. Primary endpoints: Toxicity (CTCAEv4.0). Secondary endpoints: OS, PFS, PFS6, ORR (iRANO). RESULTS: Interim analysis was based on data prior to April 1, 2018. 16 patients were screened, 1 declined, and 2 had KPS < 70. 3 patients were excluded prior to TTFields (radionecrosis/non-recurrence, post-op infection, and neurodeficit, respectively). All included patients (10, M/F 9:1) had GBM IDH-wt tumors (4 MGMT-methylated). Median baseline variables were KPS 90 (range 70–100), age 55 years (range 49 to 67), skull-defect area 10.5 cm2 (range 7 to 24), field enhancement 37 % (range 25 to 61), and TTFields compliance 91% (range 61 to 95). All patients received maximum safe resection (4 had non-measurable and 2 measurable disease). 8 patients received adjuvant bevacizumab and 2 temozolomide rechallenge. 5 patients had progression, 3 died, 5 were censored for PFS and 7 for OS. We observed no SUSARs, no grade 4/5 SAEs, 5 grade 3 SAEs (2 generalized seizures, 1 post-op infection, 1 diarrhea, and 1 DVT). 2 patients had grade 1–2 skin rash, and 1 had grade 1–2 headaches. Median outcome estimates: OS 15.5 months, 95%-CI: 5.9-NA, PFS 9.5 months, 95%-CI: 3.7-NA, and PFS6 58%, 95%-CI: 0.27–0.90. 1 patient had complete response. CONCLUSIONS: SR-surgery with TTFields is non-toxic and holds promising potential for improving rGBM outcome. A future phase 2/3 trial is being planned.

ACTR-44. PRELIMINARY RESULTS FROM THE NCT02770378 PROOF-OF-CONCEPT CLINICAL TRIAL ASSESSING THE SAFETY OF THE CUSP9v3 PROTOCOL COMBINED WITH METRONOMIC TEMOZOLOMIDE FOR RECURRENT GLIOBLASTOMA

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BACKGROUND: Despite refinements of neurosurgical techniques and emerging adjuvant therapies, patients with recurrent glioblastoma continue to face a dismal prognosis. We report preliminary results of a clinical trial evaluating a protocol of 9 repurposed drugs (aprepitant, minocyclin, disulfiram, celecoxib, sertraline, captopril, itraconazole, ritonavir, auranofin) and low-dose metronomic temozolomide in patients with recurrent glioblastoma. METHODS: Between November 2016 and April 2018, 10 patients (age 18, KPS 70%) with glioblastoma recurrence after standard therapy were included in the CUSP9v3 single-arm proof-of-concept clinical trial. The primary endpoint was dose-limiting toxicity during the first 12 weeks of treatment. Secondary endpoints were overall survival and best tumor response during the 12-month medication period according to RANO criteria. RESULTS: Fifty-five cycles of CUSP9v3 were administered. Median follow-up was 6.6 months (1.9 18.5 months) on May 30th, 2018. No drug-related serious adverse events were observed. In two patients, a permanent dose reduction (-50 % of the daily dose) was instituted for one drug (ritonavir). For eight patients, a temporary dose reduction for one to three drugs (including aprepitant, auranofin, captopril, ritonavir and/or temozolomide) was necessary so far. Five patients showed radiologic regression or stable disease. Five patients showed progression, two of which died during the first 6 weeks after inclusion. One patient died from tumor progression after completing three treatment cycles. Two patients with radiologic progression remain clinically well and continue to receive CUSP9v3 treatment. CONCLUSION: With close ambulatory monitoring and drug schedule adaptations according to individual side effects, CUSP9v3 appears to be a safe protocol with adverse effects comparable to those of more established second- and third-line treatments. Assessment of efficiency is preliminary but suggests biological activity of CUSP9v3.

ACTR-45. PHASE 0/2 STUDY OF RIBOCICLIB IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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BACKGROUND: CDK4/6-dependent cell-cycle regulation is disrupted in 78% of glioblastoma patients. We conducted a Phase 0/2 clinical trial (NCT02933736) of ribociclib, a selective CDK4/6-inhibitor, to examine the plasma and tumor pharmacokinetics (PK) and pharmacodynamics (PD) of recurrent glioblastoma. METHODS: Eligible patients with intact RB expression and CDKN2A deletion or CDK4/6 amplification were enrolled into the Phase 0 component to receive ribociclib (900mg daily) for 5 days prior to tumor resection. Plasma, tumor, and CSF samples were collected to determine drug concentrations using validated LC-MS/MS methods. PD effects, including RB and FoxM1 phosphorylation, were compared to archival tissue. Patients with favorable PK and PD outcomes were transitioned into the Phase 2 component. RESULTS: Twelve patients were enrolled into three presurgical time-escalation groups (2-hours, 8-hours, 24-hours). Ribociclib penetrated both enhancing and non-enhancing regions of the tumor. In non-enhancing tissue, median unbound brain-to-plasma ratio was 1.78 and median ribociclib concentration was 0.54 nmol/mL, exceeding the in vitro IC50 for CDK4/6 (0.04 nmol/mL). Suppression of G1-to-S phase was inferred by a decrease in RB phosphorylation ($p<0.01$) and cell proliferation ($p<0.05$). Six patients (50%) were graduated to the Phase 2 component and demonstrated a median progression-free survival of 9.7 weeks (95% CI, 6.3 to 40 weeks). Tissue samples from re-resection following Phase 2 study identified mTOR pathway upregulation as a candidate resistance mechanism to ribociclib monotherapy. CONCLUSION: Ribociclib penetrates the tumor-brain barrier, achieving pharmacologically-active concentrations in human glioblastoma and suppressing tumor proliferation. Phase 2 study results suggest ribociclib is ineffective as a monotherapy, but analysis of ribociclib-resistant tumors identified the addition of an mTOR inhibitor as a dual-drug strategy for recurrent glioblastoma.

ACTR-46. HIGHER DOSES OF TTFIELDS IN THE TUMOR ARE ASSOCIATED WITH IMPROVED PATIENT OUTCOME

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INTRODUCTION: It has been suggested that increasing Tumor Treating Fields (TTFields) dose to the tumor leads to better patient outcome.

However, this hypothesis has yet to be demonstrated. Here we present a first-of-its-kind study testing this hypothesis using data collected during the EF-14 trial that demonstrated the clinical benefit of combining TTFields with chemoradiation in newly diagnosed glioblastoma patients. METHODS: Patients treated with TTFields for at least 2 months for whom baseline MRI quality was sufficient for creation of a computational model were included in the study (n=317). The patients transducer array layouts, average compliance (fraction of therapy time), and the average electrical current derived from TTFields generator logs, were collected. Based on these data, realistic computational simulations of TTFields delivery to the patients were performed. Local Minimum Dose Density (LMiDD) was defined as the lower of the power densities delivered by two roughly orthogonal electric fields to each point in the model, multiplied by the average compliance of the patient. The average LMiDD (within a tumor bed combining the tumor and a 3 mm wide zone around it) was calculated. A value of average LMiDD that divided the patients into two groups with the most statistically significant difference in median OS was sought. RESULTS: The optimal threshold of average LMiDD was equal 1.6 mW/cm3 OS (25.1 months vs. 20.9 months, p=0.002, HR=0.69); PFS (8.8 months vs 6.5 months, p=0.006 HR=0.66). Prognostic factors in both groups were similar. The median OS and PFS were also longer when average electric field in the tumor bed was >1.05 V/cm OS (25.0 months vs. 21.6 months, p=0.043, HR=0.76) and PFS (8.1 months vs 7.9 months, p=0.006 HR=0.74). CONCLUSIONS: This study utilizes clinical data to demonstrate the connection between TTFields dose and efficacy, emphasizing the importance of TTFields treatment planning for improving patient outcome.

ACTR-47. PATIENTS WITH EGFR AMPLIFICATION BUT WITHOUT EGFRvIII EXPRESSION HAVE IMPROVED BENEFIT COMPARED TO THOSE WITH EGFRvIII EXPRESSION IN SAMPLES OF THE INTELLANCE 2/EORTC 1410 RANDOMIZED PHASE II TRIAL

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BACKGROUND: A randomized phaseII trial on EGFR-amplified recurrent glioblastomas showed that Depatux-M+TMZ improves overall survival compared to the single agent control arms. We performed targeted next-generation-sequencing to identify patients that benefit from the combination. METHODS: DNA and RNA was isolated from samples, collected at initial diagnosis, and selected for regions with highest tumor content. Target selection was done using the Trusight170 panel (Illumina). Patients were eligible with centrally confirmed EGFR amplification, defined as EGFR/CEP 7 (centromere) ratio 2 in 15% of cells (FISH). RESULTS: DNA and RNA data were generated from 233 and 234 samples respectively (of the 260 study patients). High-copy gene amplification was detected in EGFR (n=202), MDM2 (n=20), MDM4 (n=22), CDK4 (n=24) and CDK6 (n=5) which correlated with high expression levels. With this assay, 17 tumors did not show EGFR copy number (cn) aberrations (cn < 2.8), a further 14 showed copy number changes consistent with trisomy (2.8< cn < 4). Most EGFR amplified tumors also had additional genetic changes in the EGFR locus including point mutations (111/202), splice variants (132/202, the most common being EGFRvIII (n=96) or fusion genes (13/202). Response to treatment was not correlated to EGFR gene expression or amplification levels though, since EGFR amplification was a pre-requisite for inclusion, the majority of cases expressed high levels of EGFR. Preliminary analysis suggests that subjects with EGFR amplification but without EGFRvIII expression benefitted more than those with EGFRvIII expression (median survival 14.6 v 9.2 months, HR 0.57, P=0.04). No such association was identified in the control arms; median survival of EGFRvIII-negative v positive was 7.7 v 8.3 (Depatux-M) and 8.8 v 7.5 (TMZ/CCNU) months. CONCLUSION: Depatux-M in combination with TMZ confirmed the OS improved outcome in EGFR amplified recurrent glioblastoma. The improved outcome may be related to an absence of EGFRvIII expression.

ACTR-48. FEASIBILITY OF DISCONTINUATION OF ADJUVANT TEMOZOLOMIDE AFTER 12 CYCLES REMAINING WITHOUT PROGRESSION FOR PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: Standard of care for newly diagnosed glioblastoma (GBM) has been chemoradiotherapy with concomitant and adjuvant temozolomide (TMZ) (Stupp regimen). Optimal number of adjuvant TMZ cycles, however, has not been well established yet, although the pivotal trial used 6 cycles. In routine practice, up to 24 cycles have been frequently given, while many recent trials have adopted 12 cycles without solid evidence. Here we investigated outcome of and prognostic factors associated with discontinuation of adjuvant TMZ after 12 cycles without progression. METHODS: Ninety six patients treated with Stupp regimen since May 2007 to March 2017 in our institution were retrospectively analyzed. Overall survival (OS) and progression-free survival (PFS) were evaluated with Kaplan-Meier method and statistical significance was determined by logrank test. RESULTS: There were 20 patients (21%, med age 57 yo; med KPS 70; female 12) identified who completed 12 adjuvant TMZ cycles without progression. Nine patients discontinued adjuvant TMZ (Group A), while 11 continued beyond 12 cycles (Group B). Median PFS (mPFS) and median OS (mOS) were 40.6 m and 53.7 m, respectively. mPFS after 12 cycles of adjuvant TMZ (mPFS-TMZ12) for all 20 patients, Group A, and B were 26.8 m, 96.2 m, and 26.8 m (p=0.379 for latter 2), respectively. mOS after 12 cycles of adjuvant TMZ (mOS-TMZ12) were 40 m, not reached, and 27.2 m (p=0.080), respectively. Factors significantly associated with better PFS were young age, female, higher KPS, debulking resection, and no residual enhancing disease (NRD) at TMZ12, and were female, debulking resection, and NRD at TMZ12 with OS. CONCLUSIONS: A minor subset of GBM patients could continue adjuvant TMZ for 12 cycles without progression and benefited for better survival. In this cohort of patients, discontinuation of adjuvant TMZ after 12 cycles may not compromise their outcome, especially in those with no residual disease.

ACTR-49. PriCoTTF: A PHASE I/II TRIAL OF TUMOR TREATING FIELDS PRIOR AND CONCOMITANT TO RADIOTHERAPY IN NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: Tumor Treating Fields (TTFields) in combination with adjuvant temozolomide (TMZ), applied after completion of radiochemotherapy, showed significantly improved clinical outcome in newly diagnosed glioblastoma (GBM) patients in the EF-14 trial. In preclinical settings, TTFields synergistically enhanced efficacy of radiotherapy in GBM, hypothetically by inhibiting DNA damage repair in irradiated cells. The presented phase I/II trial will evaluate safety and feasibilty of TTFields initiated prior and concomitant to combined radiochemotherapy in newly diagnosed GBM. METHODS: In arm A of this prospective multi-center trial, seven eligible patients with newly diagnosed GBM will be enrolled initially. Provided that treatment is tolerated well, enrollment will continue for up to 20 patients. Patients will be subjected to TTFields after complete wound-healing following surgery. TTFields treatment will be continued throughout radiochemotherapy and adjuvant chemotherapy for six cycles. In total, patients will receive TTFields therapy for approximately nine months. In arm B, elderly patients with a reduced KPS (50 or 60) will be treated with postsurgical TTFields therapy followed by TTFields therapy concomitant to hypofractionated radiotherapy with 40 Gy for three weeks. TTFields therapy will continue throughout adjuvant chemotherapy for a total of nine months. In the first stage, six patients will be accrued. Under the provision of an acceptable safety profile, seven additional patients will be accrued. RESULTS: The primary endpoint of the trial is safety and tolerance based on the frequency of a set of predefined treatment-limiting toxicities (TLTs). Secondary endpoints consist in particular of PFS, OS, radiologic response and frequency of adverse events. First experiences will be presented. CONCLUSION: The objective of this trial is to demonstrate that the administration of TTFields therapy prior and concomitant to radiotherapy and adjuvant chemotherapy is feasible and safe. Moreover, first data obtained on efficacy may serve as a basis for a potential randomized phase III trial.

ACTR-50. EFFECT OF CONCURRENT AND ADJUVANT TEMOZOLOMIDE ON SURVIVAL IN PATIENTS WITH NEWLY DIAGNOSED GRADE III GLIOMAS WITHOUT 1p/19q CO-DELETION: A RANDOMIZED, OPEN-LABEL, PHASE 2 STUDY (INTERIM RESULTS FROM THE KNOG-1101 STUDY)

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OBJECTIVE: We investigated the efficacy between radiotherapy alone versus concurrent chemoradiotherapy with temozolomide followed by adjuvant temozolomide in Korean adult patients with newly diagnosed non-co-deleted grade III gliomas. METHODS: This was a randomized, open-label, phase 2 study, and the first multicenter, prospective clinical trial for Korean patients with glioma. Eligible patients were aged 18 years or older and had newly diagnosed grade III gliomas with ECOG performance status of 0–2. No co-deletion of chromosome 1p/19q and further genetic biomarker was identified for available subjects. Patients were enrolled at 11 centers, and randomized 1:1 to receive radiotherapy alone (daily fractions of 2 Gy given 5 days per week for 6 weeks, for a total of 60 Gy) [Control group (n=44)], or to receive radiotherapy with daily temozolomide (75 mg/m2/day, 7 days/week from the first to the last day of radiotherapy) followed by six cycles of adjuvant temozolomide (150 to 200 mg/m2/day for 5 days during each 28-day cycle) [Treatment group (n=40)]. The primary endpoint was 2-year progression-free survival (PFS). RESULTS: At the time of the preliminary analysis, PFS at 2 years was 42.2% with treatment group and 37.2% with control group. On univariable analysis, extent of tumor resection, age, and IDH-1 mutant were significantly associated with PFS. On multivariable analysis, IDH-1 mutant was the only significant prognostic factor for PFS (HR0.27; 95% CI, 0.12–0.59; p=0.001). IDH-1 mutant was also the only independent prognostic factors for overall survival (HR0.10; 95% CI, 0.02–0.41; p=0.002). Adverse events over grade 3 were seen in 16 (40.0%) patients with Treatment group, but were mild and reversible. CONCLUSIONS: Radiotherapy with concurrent and adjuvant temozolomide in patients with newly diagnosed non-co-deleted anaplastic gliomas in Korean adults trended toward improved 2-year PFS. The survival benefit of this regimen would need further analysis for long-term follow-up.

ACTR-51. PHASE 2 STUDY TO EVALUATE THE SAFETY, PHARMACOKINETICS AND CLINICAL ACTIVITY OF PI3K/MTOR INHIBITOR GDC-0084 GIVEN TO GLIOBLASTOMA (GBM) PATIENTS WITH UNMETHYLATED O_6-METHYLGUANINE-METHYLTRANSFERASE PROMOTER STATUS

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BACKGROUND: GDC-0084 is a potent, oral, selective small molecule inhibitor of class I phosphoinositide 3-kinase and mammalian target of rapamycin (PI3K/mTOR) efficacious in GBM models driven by activation of the PI3K pathway. GDC-0084 crosses the blood-brain barrier (BBB) and achieves a brain / plasma ratio of approximately 1.0 in three animal species. GDC-0084 was given as once daily oral dosing in a phase 1 study (Wen et al, J Clin Oncol 34, 2016(15) suppl.2012; NCT01547546) in 47 patients with recurrent high-grade gliomas. The adverse events were generally consistent with the established Class I PI3K/mTOR inhibitor class-effects. GDC-0084 was rapidly absorbed and demonstrated linear- and dose-proportional increases in exposure. The MTD was determined to be 45 mg once daily, with 7/8 patients receiving this dose having drug exposures consistent with anti-tumor activity in pre-clinical models. Fluorodeoxyglucose-positron emission tomography (FDG-PET) scans suggested that GDC-0084 crossed the BBB, with a uniform distribution throughout the brain. The current phase 2 study will investigate the efficacy and safety of GDC 0084 in patients with newly diagnosed GBM, with unmethylated O6-methylguanine-methyltransferase (MGMT) promoter status. METHODS: This protocol (NCT03522298) has a 2-part design. The phase 1b component consists of an open-label, multicenter dose-escalation study with expansion assess the safety, tolerability, RP2D, PK and clinical activity of GDC 0084 in patients with newly-diagnosed GBM with unmethylated MGMT. The dose-escalation portion of the study will use a standard "3 + 3" design to determine the MTDs for QD, QOD and 3 days on/4 days off schedules. At each of the identified MTDs 10 subjects will be recruited in an expansion cohort. The RP2D will be selected from the three dose schedules being tested. The phase 2 component will evaluate clinical activity of GDC-0084 at the RP2D vs temozolomide as adjuvant therapy following surgical resection and chemoradiation in 224 patients.

ACTR-52. TUMOR TREATING FIELDS (TTFIELDS) IN COMBINATION WITH LOMUSTINE (CCNU) IN THE EF-14 PHASE 3 CLINICAL STUDY – A SAFETY ANALYSIS

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INTRODUCTION: TTFields are low intensity, intermediate frequency, alternating electric fields. The significant overall and progression-free survival benefit, shown in the EF-14 phase 3 study was seen in all patient-subgroups, independent of e.g. MGMT-promotor methylation-status or age. TTFields were not associated with additional systemic toxicity. At recurrence, patients were allowed to continue TTFields with second-line therapies. Here, we analyzed the safety data of TTFields + lomustine (CCNU) to evaluate safety and feasibility of this combination. METHODS: Patients in the EF-14 trial received TTFields until second progression, or for 24 months. Change in chemotherapy regimen was allowed after tumor progression. We compared the patients who received lomustine as second-line chemotherapy in combination with TTFields (n=134) to the patients who received lomustine as monotherapy after first progression (n=39). We compared baseline characteristics and the adverse event profile between the groups. RESULTS: Baseline characteristics were well balanced except for less female patients in the lomustine only group (7.7% vs. 22.4%). Median age in the TTFields/lomustine group was 55.5 years (29–83) compared to 50.0 years (19–71) for lomustine alone. The addition of TTFields to lomustine therapy was not associated with any significant increase in systemic adverse events compared to lomustine therapy alone (number of patients with 1 SAE 30 % vs. 31 %) and the distribution, severity and overall incidence of adverse events were not statistically different in patients in the two treatment groups. CONCLUSION: The data show that the combination of TTFields and lomustine is safe and feasible. This analysis emphasizes again the strong safety profile of TTFields and the high potential of combining TTFields with other modalities. This data is especially important in light of the recently presented, promising data from a small randomized trial that tested the combination of lomustine + TMZ in newly diagnosed (MGMT promotor-methylated only) glioblastoma patients.

ACTR-53. STEAM / EORTC 1608: STUDY OF TG02 IN ELDERLY NEWLY DIAGNOSED OR ADULT RELAPSED PATIENTS WITH ANAPLASTIC ASTROCYTOMA OR GLIOBLASTOMA - A PHASE 1B STUDY

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BACKGROUND: TG02 is an oral multi-cyclin dependent kinase (CDK) inhibitor that is thought to inhibit tumor growth mainly through CDK9-dependent depletion of oncoproteins such as MCL-1 and MYC. MCL-1 and MYC are frequently overexpressed in glioblastoma (up to 80%). Several studies including analyses of patient-derived glioma cell lines have demonstrated profound inhibitory activity (IC50 range = 25–150 nM). Clinical pharmacokinetics from a phase 1b study in multiple myeloma patients demonstrate that TG02 exposures in humans are sufficient for achieving inhibitory concentrations required in the majority of the glioma cell lines tested. Preclinical studies in mice have demonstrated that TG02 is a good candidate for development in gliomas. METHODS: Based on these data, the EORTC Brain Tumor Group, in cooperation with Tragara, is currently conducting the phase 1b STEAM trial (EORTC 1608), a three parallel group (A,B,C) open-label, non-randomized, multicenter study. The recommended phase II dose of TG02 in elderly patients with IDH1R132H-non mutant newly diagnosed glioblastoma will be determined in a classical 3 + 3 design dose-escalation and safety study of TG02 in combination with either hypofractionated radiotherapy (RT) or temozolomide (TMZ) in arms A and

B. Patient allocation to treatment in arms A and B will be determined by MGMT promoter methylation status centrally determined according to EANO guidelines for the treatment of elderly patients with glioblastoma. Arm C will explore single agent TG02 activity in anaplastic astrocytoma or glioblastoma at first relapse after initial treatment with TMZ/RTTMZ with a primary endpoint of progression-free survival at 6 months. Secondary objectives include efficacy, quality of life, safety, and correlation with molecular markers. The study is currently open in 2 sites and shall be opened until SNO in 5 additional EORTC sites in Europe. Patient enrolment is planned to start in June 2018. An update will be provided at the SNO conference. NCT03224104

ACTR-54. PATTERN OF RECURRENCE AND IMPACT ON SURVIVAL OF SALVAGE THERAPIES IN LOW-GRADE GLIOMAS FOLLOWING DOSE-DENSE TEMOZOLOMIDE

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INTRODUCTION: Initial chemotherapy with temozolomide (TMZ) may provide some benefit in high-risk low-grade gliomas. No standard treatment is validated at progression. The aim of the study was to investigate which is the optimal salvage therapy after the first progression in terms of PFS and the overall survival (OS). PATIENTS AND METHODS: We evaluated 49 patients with grade II oligodendroglioma and oligoastrocytoma according to WHO 2007 included in a phase II AINO trial, who progressed following initial chemotherapy alone with TMZ. Molecular data were available in 48 patients: 29 patients were IDH 1/2 mutated, 23 1p/19q codeleted, and 36 MGMT methylated. Median follow up was 140 months. RESULTS: All patients had local tumor progression and patterns on MRI were as follows: a FLAIR lesion in 25 patients (51.0%), a mild patchy enhancing lesion in 14 (28.6%), and a nodular enhancing lesion in the other 10 (20.4%). Twenty-four patients (49%) underwent a second-line chemotherapy, 12 (24.5%) a salvage radiotherapy, 11 (22.4%) a second surgery (10 gross-total resection and 1 subtotal resection), and 2 (4.1%) palliative care. Responses (RANO criteria) following salvage radiotherapy or chemotherapy consisted in PR in 6/38 patients (15.8%), MR in 9/38 (23.7%), SD in 10/38 (26.3%), and PD in 13/38 (34.2%). Median PFS after first salvage therapy was 18 months (IC95% 11–27). Median PFS was 11 months (IC95% 8–74) after radiotherapy, 14 months after chemotherapy and 31 months after surgery (IC95% 18–51, p 0.013). Median OS from the first salvage therapy was 63 months (IC95% 4 - NR). Median OS was 44 months (IC95% 4 NR) after radiotherapy, 38 months (IC95% 22–80) after chemotherapy, and 87 months after surgery (IC95% 11-NR, p 0.09). CONCLUSIONS: Reoperation aiming at total or near/total resection seems to offer a probability of a longer PFS and OS compared with the other treatment options.

ACTR-55. TUMOR VOLUME AS A PREDICTOR OF RESPONSE TO ANTI-EGFR ADC ABT-414

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BACKGROUND: Adverse biophysical factors (abnormal vessels and increased interstitial pressure) are increased in larger brain tumors, which negatively impacts penetration of antibody drug conjugates (ADC). The tumour-specific anti-EGFR ADC, depatuxizumab mafadotin (depatux-m), demonstrated encouraging activity in a Phase 1 glioblastoma study (M12-356 study, NCT01800695). The impact of tumour size on depatux-m efficacy was investigated. PRECLINICAL STUDY: Forty mice were engrafted with patient derived xenografts from an M12-356 patient. Eight mice were imaged in a zirconium-labelled depatux-m (3mg/kg) biodistribution study at small (70mm3,n=2) or large (350mm3,n=2) tumour volumes; another 4 mice were imaged with a control ADC. In the controls, non-specific uptake due to blood pool activity was 5% ID/g. In the 89Zr-depatux-m treated groups, mice with larger tumours had significantly less uptake compared smaller tumours (11 vs 21 %ID/gram, p<0.0001). Similarly, tumour inhibition due to depatux-m (3mg/kg every 3 days for 3 weeks, n=16 mice) was significantly less when treatment commenced at larger volumes (27% vs 93%, p<0.001). CLINICAL STUDY: To confirm our preclinical data, two reviewers (EL, AS) retrospectively undertook a blinded volumetric analysis of baseline tumor volumes in M12-356. Inter-rater agreement was excellent. Response was compared by chi-square analysis and OS by log-rank. 110 patients with EGFR-amplified, recurrent glioblastoma were treated with depatux-m, alone or in combination with temozolomide. Patients with larger tumours had significant worse response rates (0% in > 25cm3 vs 17% in < 25cm3, p=0.009) and worse overall survival (0.52 vs 0.81 years, p=0.001). The results were similar in patients treated with depatux-m monotherapy for response (n=60, 0% vs 10%, p=0.287) and survival (n=62, 0.50 vs 0.89 years, p=0.001) CONCLUSIONS: Increased tumour volumes results in significant reduction in ADC penetration. The impact of this as a modifiable factor, within the broader prognostic impact of increased tumour volume, warrants further investigation with prospective and/or randomized data.

ACTR-57. RELATIVE CEREBRAL BLOOD VOLUME (RCBV) AS RESPONSE PREDICTOR IN THE TREATMENT OF RECURRENT GLIOBLASTOMA (GB) WITH ANTIANGIOGENIC THERAPY

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INTRODUCTION: Glioblastoma (GB) is the most common primary brain tumor in adults having an overall survival (OS) ranging from 12–14 months. GB is highly vascularized, partly due to excessive levels of VEGF. Recurrence is the rule in GB, the second line of treatment is antiangiogenics of which Bevacizumab(BVZ) - a humanized monoclonal antibody to the VEGF-A - is the most commonly used antiangiogenic agent in the setting of recurrent glioblastoma (rGB). rCBV has been evaluated as a predictor of response to this therapy using perfusion-weighted MR techniques. METHODS: Patients with rGB according to RANO criteria treated with BVZ with an MRI perfusion with rCBV prior treatment initiation were enrolled. The correlation between rCBV and response was analyzed by correlation coefficient. A cut-off point to identify responders and non-responders to BVZ was determined by ROC curve and OS was evaluated using Kaplan Meier curve and log rank. RESULTS: 31 patients with GB were included. We divided the patients in non-responders (n=17) and responders (n=14), there were differences between the groups (age and KPS). The correlation between the RCBV and the Therapeutic response was r=-0.83, p0.0001 (95% CI -0.918 to -0.675). The ROC curve showed 3.7 as the cutoff value with a sensitivity of 100% and specificity of 94% to predict response to BVZ. We divided the groups into non-responders and responders, with OS of 7.7 months vs 14.4 months respectively, p 0.045 (HR 2.3, 95% CI 1.0192 to 5.6192). CONCLUSIONS: rCBV is a potential biomarker evaluated by MRI that predicts response to treatment in patients who will be treated with Bevacizumab. Bevacizumab treatment is highly cost-effective, and this biomarker would help identify patients that would benefit. This biomarker as predictive/prognostic has been widely used and is currently the most reliable parameter for measuring the response to antiangiogenic treatment.

ACTR-58. TREATMENT OUTCOMES OF STEREOTACTIC RADIOTHERAPY COMBINED WITH BEVACIZUMAB THERAPY IN RECURRENT GLIOBLASTOMA PATIENTS

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BACKGROUND: The prognosis for patients with recurrent glioblastoma is still poor with a median survival between 3 and 6 months. METHODS: We performed a retrospective analysis of 11 patients with recurrent glioblastoma treated with stereotactic radiotherapy (25Gy/5fr) followed by 200 mg/m2 of Bevacizumab every three weeks. Overall survival was estimated by the Kaplan-Meier method. RESULTS: The median age of the group was 59 years. Median overall survival from initial diagnosis was 21 months weeks. Median overall survival from recurrence was 11 months. No adverse events (National Cancer Institute Common Terminology Criteria for Adverse Events grade 3 or 4) occurred in any patient. CONCLUSION: Stereotactic radiotherapy combined with BEV treatment was found to be safe and effective in recurrent glioblastoma patients.

ACTR-59. IMPROVING THE INTRA-OPERATIVE DIAGNOSIS OF HIGH-GRADE GLIOMA USING A FLUORESCENCE BIOMARKER – RESULT OF THE GALA-BIDD STUDY

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BACKGROUND: Correctly distinguishing gliomas as low or high grade (LGG or HGG) during surgery can influence the surgical procedure, enhancing resection and improving survival. The UK NCRI GALA-BIDD study was designed to prospectively investigate whether the presence of visible fluorescence is a pragmatic intra-operative diagnostic surgical biomarker of high-grade disease within a tumour mass in real time during surgery. METHODS: Patients with suspected intrinsic glioma discussed at neuro-oncology Multidisciplinary Team meetings and suitable for fluorescence guided cytoreductive surgery were eligible. 5-aminolevulinic acid (5-ALA) was used to generate visible fluorescence. Samples of fluorescent tissue were sent for peri-operative histopathological analysis to establish an intra-operative diagnosis of LGG or HGG. These data were compared with the final central pathological diagnosis. RESULTS: From Feb 2015 to March 2017 in the UK, 106 patients were recruited: median age 59 (range 23–77); 59% male; 25% WHO radiological grade II transforming to a higher grade and 55% grade IV. 5-ALA were given for 103 patients with a median dose of 1500mg (range 960-2200mg). 67% of patients classified as HGG at local peri-operative diagnosis were confirmed by the central review (weighted Kappa 0.37 (95%CI=0.21–0.54)). 88 patients were evaluable for the primary endpoint: 81 had visible fluorescence of the tumour with central histopathology diagnosis as 1 LGG, 78 HGG (a 99% concordance in HGG classification with the 99%CI=91%-99.9%) and 2 not assessed; 7 patients had no visible fluorescence and were diagnosed as 6 LGG and 1 HGG. CONCLUSION: There is an urgent need to improve the local peri-operative diagnosis. The presence of visible fluorescence can be used as an additional pragmatic intra-operative diagnostic surgical biomarker of high-grade disease within a tumour mass. Use for assessment of low-grade disease needs further investigation.

ACTR-61. LONG-TERM ANALYSES OF THE NOA-08 RANDOMIZED PHASE III TRIAL

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BACKGROUND: Optimal treatment and role of O6-methylguanine DNA-methyl transferase (MGMT) status in elderly patients is still not defined. METHODS: This is the long-term update (LT) of the NOA-08 trial (NCT01502241) that compared efficacy and safety of RT to TMZ in elderly patients with anaplastic astrocytoma (AA) or glioblastoma (GB) using overall survival (OS) as primary endpoint and event-free survival (EFS) as well as efficacy according to MGMT status as major secondary endpoints. FINDINGS: In the LT with a data cut-off Apr 1 2018 median OS was 8·2 [7·0–10·0] months for TMZ treatment versus 9·4 [8·1–10·4] months for RT; hazard ratio (HR)=0·93 (95% CI: 0·76-1·15)] of TMZ versus RT did not differ between both arms. Also, median EFS [3·4 [3·2–4·1] months versus 4·6 [4·2–5·0] months 3did not differ with a HR=1·02 (0·83-1·25)]. MGMT promoter methylation tested in tumor tissue (82/221 patients, 37·1%) was associated with prolonged OS [13·6 [10·1–16·5] versus 8·0 [6·9-9·9] months; HR=0·53 (0·40-0·70), p<0·0001]. Patients with MGMT promoter methylation had longer OS and EFS when treated with TMZ (18·4 [13·9–24·4] months and 8·5 [6·9–13·3] months) versus RT (9·6 [6·4–13·7] months and 4·8 [4·3–6·2] months, HR 0·44 [0.27–0.70], p<0·001 for OS and 0·46 [0.29–0.73], p=0·001 for EFS). Patients without MGMT promoter methylation had shorter EFS and a shorter OS with the usual testing not significant when treated with TMZ (6·7 [5·6–8·2] months and 3·0 [2·6-3·3] months) versus with RT (10·2 [8·0–12·0] months and 4·6 [3·7-6·4] months), HR 1·33 [0·95-1·87], p=0·099 for OS and 1·86 [1·32-2·62], p<0·001 for EFS). INTERPRETATION: LT of NOA-08 confirms the non-inferiority of TMZ compared with RT in the treatment of elderly patients with AA or GB. To improve OS and EFS, MGMT promoter methylation is a strong predictive biomarker for the choice between RT and TMZ and offers unexpectedly favorable long-term outcome with initial TMZ monotherapy.

ACTR-62. PHASE I/II STUDY OF TEMOZOLOMIDE PLUS NIMUSTINE CHEMOTHERAPY FOR RECURRENT MALIGNANT GLIOMAS: KYOTO NEURO-ONCOLOGY GROUP

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The objective of this phase I/II study was to examine the efficacy and toxicity profile of temozolomide(TMZ) plus nimustine (ACNU) Patients who had received a standard radiotherapy with one or two previous chemo-regimens were enrolled. In phase I, the maximum-tolerated dose (MTD) by TMZ (150mg/m2/day) (Day1-5) plus various doses of ACNU (30,35,40,45 mg/m2/day) (Day15) per 4 weeks was defined on a standard 3 + 3 design. In phase II, these therapeutic activity and safety of this regimen were evaluated. Forty-nine eligible patients were enrolled. The median age was 50 years-old. Eighty percent had a KPS of 70100. Histologies were glioblastoma(73%), anaplastic astrocytoma(22%), anaplastic oligodendroglioma(4%). In phase I, 15 patients were treated at four cohorts by TMZ plus ACNU. MTD was TMZ (150/mg/m2) plus ACNU (40mg/m2). In phase II, 40 patients were treated at the dose of cohort 3 (MTD). Thirty-five percent of patients experienced grade 3 or 4 toxicities, mainly hematologic. The overall response rate was 11% (4/37). Sixty-eight percent (25/37) had stable disease. Twenty-two percent (8/37) showed progression. Progression free survival (PFS) at 6 and 12 month were 24% (95%CI, 12–35%) and 8% (95%CI, 4–15%). Median PFS was 13 months(95%CI, 9.2–17.2months). Overall survival (OS) at 6 and 12 were 78% (95%CI, 67–89%) and 49% (95%CI, 33–57%). Median OS was 11.8 months (95%CI, 8.2–14.5months). This phase I/II study showed a moderate toxicity in hematology and may has a promising efficacy in OS, without inferiority in PFS.

ACTR-63. TREATMENT AND SURVIVAL OF PATIENTS WITH LOWER GRADE GLIOMA ACCORDING TO THE 2007 AND THE 2016 WHO CLASSIFICATION: A RETROSPECTIVE ANALYSIS OF 423 PATIENTS

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INTRODUCTION: Classification as well as treatment of patients with lower grade gliomas (°II+°III) have changed fundamentally during the last years. Molecular markers have augmented diagnostic workup and combined radiochemotherapy was established for most of the subgroups. However, molecular markers have not been part of the inclusion criteria of most of the relevant clinical trials. Larger analyses outside of clinical trials are rare. MATERIALS AND METHODS: We screened our clinical cancer database for patients with lower grade glioma newly diagnosed from 1995 to 2015. We identified 774 patients of whom 345 had to be excluded, resulting in an evaluable cohort of 423 patients. We evaluated general characteristics, morphological diagnosis, molecular markers, treatment, time-to-treatment-failure (TTF; initiation of a new treatment or death) and overall survival (OS). RESULTS: According to the 2007 WHO classification our cohort included 145 (34.3%) Astrocytoma WHO °II, 56 (13.3%) Oligoastrocytoma/Oligodendroglioma WHO °II, 129 (52.5%) Astrocytoma WHO °III and 93 (22.0%) Oligoastrocytoma/Oligodendroglioma WHO °III. In 235 patients we were able to molecularly classify the tumors based on the 2017 WHO classification using IDH status and 1p/19q or ATRX status. Patients with a molecularly defined Oligodendroglioma showed a median TTF of 5.2, 4.2 and 7.8 years for radiotherapy, chemotherapy and radiochemotherapy, respectively. Patients with a molecularly defined Astrocytoma showed a median TTF of 1.6, 2.9 and 6.7 years for radiotherapy, chemotherapy and radiochemotherapy, respectively. In IDH wildtype tumors TTF was below 12 months without relevant differences. Treatment with combined radiochemotherapy resulted in markedly improve TTF in molecular defined oligodendroglioma and astrocytoma compared with either radiotherapy or chemotherapy alone. Due to the short follow-up of 5.3 years (mean) median OS has not been reached for any of the IDH

mutant subgroups. CONCLUSIONS: Combined treatment with radiotherapy and chemotherapy resulted in markedly improved TTF in patients with molecularly defined oligodendroglioma and astrocytoma.

ACTR-64. OBJECTIVE RESPONSES TO CHEMOTHERAPY IN RECURRENT GLIOMA DO NOT PREDICT BETTER SURVIVAL: A PROSPECTIVE ANALYSIS FROM THE GERMAN GLIOMA NETWORK

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Outside of clinical trials, the occurrence of objective responses (OR) to chemotherapy in patients with recurrent gliomas is poorly characterized. Further, the predictive value of OR for PFS and OS is unclear. We screened the German Glioma Network Database for patients who had received chemotherapy only for recurrent glioma. As PFS was not available for a large number of patients we used the composite endpoint time-to-treatment-failure (progressive disease, start of a new therapy or death). We included 485 patients who received 646 chemotherapy regimens for treatment of recurrent glioma. Of these, only 32 chemotherapies in 32 patients resulted in an OR (30 PR, 2 CR) after central review according to RANO criteria. OR rates were 2.8%, 11.0% and 10.6% for glioblastoma, anaplastic glioma WHO grade III and diffuse glioma WHO grade II, respectively. Temozolomide (n=232) resulted in ORs in 7.8% of the patients, while nitrosourea (n=212) and imatinib (n=27) resulted in ORs in 6.1% and 3.7%, respectively. Overall, responders showed a significantly improved OS compared to non-responders (median OS 33.3 versus 15 months, p=0.054). Yet, a Cox regression analysis adjusted for diagnosis and age did not reveal a significant association of objective responses with overall survival (relative risk 0.8, p=0.391). In addition, we generated a 1:3 cohort (n=96) of non-responders matched for diagnosis. There was no relevant difference in OS comparing responders to the matched cohort (median OS 33.3 versus 25.6 months, p=0.929). When comparing non-responders with longer time-to-treatment-failure (>14 weeks, assumed stable disease as best response) with responders (n=32) the difference in outcome was lost (median OS 24.3 versus 33.3 months, p=0.86). In this prospective trial ORs to chemotherapy in the recurrent setting were rare. Notably, when comparing responders with a matched cohort or patients with assumed stable disease as best response, ORs were not associated with improved survival.

ACTR-65. INTRATHECAL PEMETREXED FOR PATIENTS WITH RECURRENT LEPTOMENINGEAL METASTASIS FROM LUNG ADENOCARCINOMA: A PHASE I CLINICAL TRIAL (IPRLM,NCT03101579)

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BACKGROUND: To determine toxicities, maximally tolerated dose (MTD), and potential antitumor activity of intrathecal pemetrexed (IP). MATERIAL AND METHODS: Pulmonary adenocarcinoma patients with recurrent or progressive leptomeningeal metastases (LM) after intrathecal chemotherapy and other LM-related therapy were included. IP doses escalated from 10 mg to 15 mg, and even 20 mg. Protocol schema of IP was twice per week for 2 weeks (induction therapy), followed by once per week for 2–4 weeks (consolidation therapy). The primary endpoints were MTD and safety. The secondary endpoint was efficacy. Plasma and cerebrospinal fluid (CSF) samples were collected and analyzed for drug concentration. RESULTS: Nine patients (male: 3; female: 6; age: 37–71 years; median: 55) were enrolled between March 2017 and March 2018. All cases received total 50 times of IP (2–8 times, median: 6). Incidence of >grade III adverse events was 50%, including 4 cases with hematological toxicities and 2 with radiculitis. One patient received 15 mg IP dose expired due to hematological toxicities. Then protocol was revised. The dose was decreased to 10 mg. B12 and folic acid supplementation was indispensable. Three more cases were enrolled subsequently. No case showed severe hematological toxicities. Total clinical response rate was 80%. For the cohort of 10 mg dose, clinical response rate was 88%. All cases were followed up until May 1, 2018. Six cases died from cancer or related complications. No acute or subacute CNS toxicity was observed. The median overall survival was 2.5 (0.3–12) months from enrollment of this study. Pemetrexed was not cumulative in CSF. Times of plasma concentration peak were 6 h in 3 cases, 9 h in 1 case, and 12 h in 1 case after IP. CONCLUSION: Pemetrexed is suitable for intrathecal use. A dose of 10 mg shows well safety and high clinical response rate, which is worth for subsequent trials.

ACTR-66. BEVACIZUMAB THERAPY FOR THE TREATMENT OF ADULT GLIOBLASTOMA: SYSTEMATIC REVIEW & META-ANALYSIS

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INTRODUCTION: Glioblastoma is the most common high-grade primary brain tumor in adults. Standard multi-modality treatment of glioblastoma often results in transient tumor control, but inevitably gives way to disease progression. The need for other therapeutic avenues led to interest in the anti-angiogenic therapy, namely bevacizumab, as a treatment for glioblastoma. We sought to determine the efficacy of bevacizumab as a treatment for glioblastoma. METHODS: We conducted a literature search using the PubMed database and Google Scholar to identify randomized controlled trials (RCTs) since 2014 investigating the safety and efficacy of bevacizumab in the treatment of adult patients (18 years and older) with both newly diagnosed and recurrent glioblastoma. Only Level I data that reported progression-free survival (PFS) and overall survival (OS) were included for analysis. RESULTS: We identified 14 studies that met our criteria, reporting on a total of 3,192 patients. Our preliminary analysis finds that treatment with bevacizumab consistently prolongs PFS with a correspondent decrease in PFS hazard ratio (HR) for treatment groups that include bevacizumab versus those that do not. Bevacizumab had no significant effect on OS in patients with newly diagnosed or recurrent glioblastoma. Seven studies reported on MGMT status: four studies found that patients with methylated MGMT status had a consistently longer PFS and OS, corresponding with significantly lower HR for both variables, when compared to unmethylated groups. CONCLUSIONS: Our preliminary findings suggest that bevacizumab therapy is associated with a longer PFS in adult patients with glioblastoma, however, bevacizumab had an inconsistent effect on OS in this patient population. The differential response to bevacizumab in relation to MGMT methylation status and molecular subtype requires further analysis.

ACTR-67. A PHASE I STUDY OF CONVECTION-ENHANCED DELIVERY OF LIPOSOMAL-IRINOTECAN (ONIVYDE) USING REAL-TIME IMAGING WITH GADOLINIUM IN PATIENTS WITH RECURRENT HIGH GRADE GLIOMAS: RESULTS THUS FAR

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BACKGROUND: Chemotherapy for high grade gliomas (HGG) is limited by the blood-brain-barrier (BBB). Convection enhanced delivery (CED) improves chemotherapy delivery by utilizing fluid convection obviating the challenges of crossing the BBB while minimizing systemic toxicity. CED of nanoliposomal-irinotecan (Onivyde) showed to be a superior delivery route for anti-tumor activity in animal models. An advance of this trial is the development and use of real time CED, which utilizes MRI to visualize the CED process with the aid of co-convected contrast agents, monitoring delivery into the brain and affording for corrective action. METHODS: This is a 3 + 3 single dose escalation trial with 2 cohorts: 20mg/ml and 40mg/ml. Onivyde and GAD were co-infused via the same catheters in a one-time delivery. The total volume of infusate, and consequently total dose, were personalized based on the patient's tumor volume, and ranged from 20–680 mg of Onivyde, given via up to 4 catheters. Tumor diameters were allowed to be 1 – 4 cm, with injection volumes ranging from 2 – 17 mL of infusate. RESULTS: 10 patients have been treated on this protocol, all in under 5 hours. There were 7 GBs, 2 anaplastic astrocytomas, and 1 oligoastrocytoma. Seven patients lived

over a year after treatment, which is remarkable since median survival rates for multiply recurrent: GBs = 8 months, AAs = 11 months. Utilizing imaging software, we correlated pre-infusion modeling of the drug distribution with post-infusion imaging. A number of technical challenges were overcome by real time monitoring; the total volume of distribution (Vd), and the Vd to volume infused (Vi) ratio for each infusion was ~2. CONCLUSIONS: Image-guided distribution allows for safe real-time placement and adjustment of CED cannula of Onivyde into patient's brains. Such methods allow for maximum tumor coverage and warrant further studies with repeat dosing.

ACTR-68. INITIAL TREATMENT OF GBM WITH A KETOGENIC DIET ALONG WITH RADIATION AND CHEMOTHERAPY: FEASIBILITY, TOXICITY AND RESPONSE

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INTRODUCTION: We initiated and now report early outcomes of a pilot clinical trial study using a ketogenic diet (KD) combined with radiation and chemotherapy as initial therapy for aggressive gliomas (glioblastoma, GBM). METHODS: Eligibility criteria included: 1. Tissue diagnosis; 2. Age over 18; 3. ECOG performance status of 2 or better; and 4. Not having diabetes or being pregnant. An isocaloric KD diet with 3:1 weight ratio of fat to combined weight of carbohydrate and protein was supervised by an experienced dietitian. Per protocol the KD together with radiation and chemotherapy was maintained for 6 weeks. RESULTS: 9 patients (mean age 45, 8F,1M) completed 6 weeks of this trial. Blood counts, chemistries, lipid profiles and uric acids were not markedly changed. No other significant side effects were observed. Ketosis was maintained for the 6 week study period with blood ketone and glucose levels checked twice daily: The ketones ranged between 0.9 and 4.1 and glucose ranged between 80.3 and 120. Patients' daily AM weights decreased on average < 10% during the study. 3 patients (mean age 28) showed no progression of their gliomas at 34 (IDH +), 19 (IDH +), and 25 (IDH WT) months since diagnosis. 6 patients (mean age 54) have died at 25, 25, 13, 12, 9 and 9 months after diagnosis. CONCLUSIONS: 1. Combining an adjuvant KD with standard therapy of radiation and chemotherapy right after tissue diagnosis is feasible and safe. 2. Daily measurements of blood ketones and glucose are necessary to assure continued ketosis; 3. KD may be a helpful adjuvant initial treatment of GBM patients, especially in younger patients. A larger study will be required to test this hypothesis.

ACTR-69. BLOOD DERIVED EXOSOMAL hTERT mRNA - A POTENTIAL BIOMARKER FOR GLIOBLASTOMA

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BACKGROUND: Primary glioblastoma multiforme (GBM) have a high proportion (~80%) of TERT-expressing tumors, indicating that this is the primary mechanism of telomerase (hTERT) activation. Exosomes are nano-sized secreted vesicles containing nucleic acids and proteins, which reflect the content of the mother cells. We have previously shown that hTERT transcripts in serum exosomes may serve as a "pan-cancer" diagnostic method, reflecting the load of hTERT in systemic cancer cells. The goal of the current study was to evaluate whether exosomal hTERT may serve as a circulating biomarker for GBM. METHODS: hTERT mRNA levels were determined in serum derived exosomes obtained from 20 GBM patients and 45 healthy controls. The level of exosomal hTERT mRNA was measured prior to surgery in all GBM patients. In 10 patients additional longitudinal evaluation was performed in blood samples obtained prior to and after concurrent radio/chemotherapy and again during adjuvant temozolomide treatment. RESULTS: Circulating hTERT transcripts were absent in controls and were variably detected in 40% of GBM patients with significantly elevated mean level at diagnosis (p=0.049). These transcripts were gradually downregulated on longitudinal evaluations and during treatment period with significant reduction observed after 3 months of adjuvant treatment when compared to initial sampling (p=0.026). In 10 patients an analysis of *hTERT* promoter mutation was performed and in 8/10 one of the two common mutations (C228T and C250T) was detected on tumor samples. CONCLUSIONS: hTERT mRNA levels may reflect the tumor burden and the clinical status of patients with GBM. In systemic tumors exosomal hTERT mRNA expression is detected in about 60% of cases while in GBM the percentage is probably lower. In patients with detectable levels, this assay may serve as a serum biomarker. These results warrant further confirmation and an update on an extended cohort of patients will be presented at the meeting.

ACTR-70. INCREASING SURVIVAL IN BIOPSY-ONLY GBM PATIENTS

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It is generally expected that survival from GBM is correlated to the extent of resection. That is, patients having only a biopsy don't live as long as those having partial or complete resections. Fifteen years ago, 2-year survival of biopsy-only patients was 5%. In 2005, Stupp *et al.* added temozolomide (TM Z) to radiation therapy and also established a follow-up TMZ chemotherapy regimen. This increased 2-year survival of unresected GBM patients from 4.6% to 10.4 % (Stupp et al., 2009). Lately, due to the still-low survival rate, biopsy-only patients are often excluded from GBM clinical trials. It is known that hypoxic tumors are resistant to radiation therapy (Sheehan *et al.*, 2010). Thus, Diffusion Pharmaceuticals Inc. added trans sodium crocetinate (TSC) to temozolomide-radiation therapy, but not to the chemotherapy in a Phase 2 clinical trial (Gainer, *et al.*, 2017). TSC stimulates re-oxygenation of hypoxic tumors (Sheehan, et al, 2009, 2011). It acts systemically to re-oxygenate hypoxic tissue but has no effect on normal cells. When combined with temozolomide and radiation, TSC resulted in a 2-year survival of biopsy-only patients of 40%, in effect quadrupling the 2005 Stoop results. TSC is being currently studied in a Phase 3 GBM trial involving solely biopsy-only patients. In this trial, TSC is added to both the radiotherapy and chemotherapy sessions, preceding the dosing of TMZ. This trial is called INTACT (NCT03393000) and is currently enrolling patients.

ACTR-71. PHASE 1/2 STUDY OF DIANHYDROGALACTITOL (VAL-083) WITH RADIATION THERAPY IN PATIENTS WITH NEWLY DIAGNOSED, MGMT-UNMETHYLATED GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and aggressive primary brain cancer. Current standard-of-care includes surgery followed by chemo-radiation and temozolomide. An unmethylated promoter for O6-methylguanine-DNA-methyltransferase (MGMT) is a validated biomarker for temozolomide-resistance and is strongly correlated with poor outcomes. Unmethylated MGMT represents the majority of newly diagnosed GBM tumors. VAL-083 is a first-in-class bi-functional DNA-targeting agent that has shown activity against GBM in NCI-sponsored clinical trials both as single agent and in combination with radiotherapy. VAL-083 induces interstrand cross-links at N7-guanine, leading to DNA double-strand breaks and cell-death. VAL-083s unique mechanism-of-action circumvents MGMT-mediated chemoresistance, and it has demonstrated cytotoxicity in MGMT-unmethylated GBM cell-lines, cancer stem cells (CSCs) and in vivo models. Furthermore, VAL-083 acts as a radiosensitizer in GBM CSCs and non-CSCs. We completed a dose-escalation trial of VAL-083 in recurrent GBM, and a generally well-tolerated dosing regimen was selected for further clinical development. The present trial is an ongoing open-label, biomarker-driven, Phase 1/2 study to evaluate the tolerability and efficacy of VAL-083 in combination with radiotherapy in newly diagnosed MGMT-unmethylated GBM patients. A treatment regimen, consisting of a 6-week induction period of VAL-083 and concurrent radiation (2 Gy daily, 5 days/week) followed by up to 24 weeks of maintenance therapy with single-agent VAL-083, is being evaluated. The study is being conducted in two parts: 1) a dose-escalation part (20, 30, and 40mg/m2/day IV infusion on days 1,2,3 of a 21-day cycle) in up to 10 patients; 2) an expansion part in up to 20 additional patients at the determined well-tolerated dose. Tumor response will be assessed by MRI, according to RANO criteria. Efficacy endpoints include progression-free survival (PFS) and overall survival (OS). Additional endpoints include safety evaluations and pharmacokinetic assessments of plasma and CSF samples. Enrollment and safety data update will be provided at the meeting. Clinicaltrials.gov identifier: NCT03050736.

ACTR-72. IDH-WILD TYPE GRADE II GLIOMAS: A RETROSPECTIVE SERIES OF ITALIAN ASSOCIATION OF NEURO-ONCOLOGY

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BACKGROUND: Information regarding clinical characteristics and response to treatments of IDH-wild type grade II gliomas are still lacking. MATERIAL AND METHODS: We performed a retrospective study on patients with WHO grade II IDH wild type gliomas recruited from 1999 to 2017 in six Italian Institutions. IDH mutation was assessed by either immunohistochemistry or sequencing (in case of negative immunohistochemistry). Exclusion criteria were the presence of minimal anaplastic foci or radiological features of HGGs. Kaplan-Meier curves and Cox-regression models were used for univariate and multivariable analysis. RESULTS: Out of 194 patients 122 met the inclusion criteria. Median age was 45 years. Non-enhancing tumours on MRI accounted for 74% while 26% had mild contrast enhancement. Surgery consisted in gross total resection in 29%, partial/subtotal in 45%, biopsy in 24%, and unknown in 2%. According to WHO 2007 astrocytomas were 44%, oligodendrogliomas 35%, and oligoastrocyomas 21%. Post-surgical management consisted in observation with MRI in 42%, chemoradiation in 21%, chemotherapy alone in 21%, radiotherapy alone in 5%, radiotherapy followed by chemotherapy in 5%, and 6% unknown. Median time of follow-up was 31 months. Median PFS at first recurrence from diagnosis was 24.0 months (1.2 147.0). Treatments at recurrence consisted in second line chemotherapy in 33 cases (42.3%), second surgery in 18 cases (23.1%), radiotherapy in 10 cases (10.4%), and palliative care in 13 cases (16.7%). Median OS was 45.3 months (1.0 225.6). Factors associated with longer PFS and OS in univariate analysis were younger age, absence of contrast enhancement, and gross total resection. Younger age and gross total resection retained the statistical significance in multivariable analysis. CONCLUSIONS: WHO grade II IDH-wild type gliomas have a worse outcome as compared with IDH-mutant tumours. This is the first real life study reporting that gross total resection improves the outcome.

ANGIOGENESIS AND INVASION

ANGI-01. CORRELATION BETWEEN HISTOLOGICAL AND MRI MEASURES OF INFILTRATION IN A PRECLINICAL GLIOBLASTOMA MODEL

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INTRODUCTION: Maximal safe cytoreductive surgery is the therapeutic ideal in GBM. Occult, non-enhancing, infiltrative disease ultimately drives treatment failure. Diffusion-weighted imaging (DWI) assessment of tumor margins through apparent diffusion coefficient (ADC) transition coefficients shows promise in non-invasive molecular subtyping and relating potentially-invasive behavior to survival. ADC non-specificity limits biomarker interpretation. We aimed to explore histological tumor cell burden relationship to ADC measurements at the macroscopic margin in a GBM model. METHODS: G7 GBM orthotopic xenografts were implanted into nude mice. T2-, and diffusion-weighted images were acquired at 7T. Brains were extracted and fresh-frozen to minimise fixation artefacts and preserve morphology. Cryosections were stained with human leucocyte antigen (HLA) marker specific for human-derived GBM cells. Confocal HLA photomicrographs (10x) and high resolution T2w MRI were linearly co-registered and resampled to match DWI. Macroscopic tumour margin was visually delineated using the low ADC/high-T2w-signal region. Linear profiles perpendicular to these boundary voxels produced serial, centrifugal, spatially-congruent measurements of ADC and HLA across the infiltrating edge over 0.7mm. Spearman's rho was calculated in each subject for every profile. RESULTS: 5 mice were suitable for analysis. In 72% of tumor boundaries (n=562), there was a statistically significant ($p<0.05$) inverse correlation (<-0.8) between ADC and HLA. Areas with insignificant/positive correlation represented distinct physical boundaries such as pia/ependyma or artefact. DISCUSSION: Characterizing microinvasive disease which ultimately leads to treatment failure in maximally-resected GBM is challenging. Research dwells on the enhancing disease which is excised. Margin assessment provides an opportunity to characterize tumor behavior at the crucial interface between dividing bulk tumor and the parenchymal infiltration which predicates recurrence. The spatially-consistent inverse correlation between tumor margin HLA and ADC values for almost all profiles at the tumor-parenchyma interface indicates that ADC transition profiles relate to tumor infiltration as previously hypothesised, with lower ADC related to higher tumor burden.

ANGI-02. A CRITICAL ROLE FOR LARG IN RhoC MEDIATED GLIOBLASTOMA CELL INVASION

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Glioblastoma (GBM) is the most common and highly lethal central nervous tumor in adults. A significant hurdle to effective clinical treatment is the aggressive, highly diffuse infiltration of tumor cells into the normal brain parenchyma which makes surgical removal of GBM tumors impossible, increases resistance to chemotherapy and radiation treatment, and ultimately leads to tumor recurrence. A distinguishing feature of the brain parenchyma is the tight extracellular space resulting from the densely packed neurons and glial cells. Invasion of glioblastoma cells into the brain parenchyma is challenged by migration through extracellular spaces that are narrower than the nuclear diameter. In an effort to identify signaling elements involved in cell invasion which requires nuclear squeezing, we examined the role of proteins involved in regulating actomyosin contractility. We demonstrate that siRNA-mediated depletion of the leukemia-associated Rho guanine nucleotide exchange factor (LARG), which was originally identified as a result of chromosomal translocation in acute myeloid leukemia, impaired the nuclear squeezing of glioblastoma cells *in vitro* and invasion into brain slices *ex-vivo*. Moreover, depletion of LARG inhibited serum-induced activation of RhoC, but not RhoA. In addition, transwell migration assays with 3-μm pore size filter and matrigel invasion assays demonstrated that RhoC is essential for nuclear squeezing and glioblastoma invasion whereas RhoA is dispensable. Finally, immunohistochemistry analysis demonstrated that the expression of LARG and RhoC increases with glial tumor grade and are highest in glioblastoma and their expression is enriched in the invading cells. Collectively, these results suggest that LARG plays an essential role in glioblastoma cell invasion and may provide new insight into targeting invasive glioblastoma cells.

ANGI-03. PSA-NCAM IN GLIOBLASTOMA – A NEGATIVE PROGNOSTIC MARKER AND A THERAPEUTIC TARGET?

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BACKGROUND: Glioblastoma (GBM) is the most common and aggressive primary brain tumour in adults. Despite the current clinical management of surgical resection, followed by radiation and chemotherapy, the median survival time is still a dismal 15 months. This may be due to treatment-resistant GBM stem cells (GSCs) that survive and migrate, resulting in tumour recurrence. Therefore, elucidating the mechanisms of GSC migration and studying ways of limiting this process will be beneficial in the treatment of GBM. A key molecule of interest is polysialylated neural cell adhesion molecule (PSA-NCAM). During normal development and adult neurogenesis, its presence on the cell surface reduces interactions with the extracellular matrix and aids in cellular migration. However, it has also been associated with malignant brain tumours and with negative patient prognosis. METHODS: We investigated PSA-NCAM's role in tumour recurrence by immunohistologically evaluating over 160 surgically resected brain tumour specimens for PSA-NCAM expression and correlating this with patient outcomes. We also utilized GSCs isolated from the same patient specimens to investigate the role of PSA-NCAM in tumour cell migration. RESULTS: Univariate and Cox proportional hazard analysis showed that PSA-NCAM expression was a strong predictor of rapid tumour progression, even more so than the cell proliferation marker Ki67, which is often used to assess tumour grade. In addition, high-content image analysis revealed that PSA-NCAM was highly expressed by migrating patient-derived GSCs *in vitro*, and drugs that limited their migration also decreased PSA-NCAM expression. This led to investigations that aimed to elucidate the effects of PSA-NCAM removal on GSC migration. SUMMARY: Our work highlights the potential role of PSA-NCAM in GSC migration and

tumour progression. This opens up avenues to pharmacologically and genetically manipulate PSA-NCAM to reduce GBM cell migration and curb tumour recurrence.

ANGI-04. TEAD1 REGULATES CELL MIGRATION IN HUMAN GLIOBLASTOMA IN PART THROUGH EMT-ASSOCIATED CADHERINS

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The epithelial to-mesenchymal transition (EMT) is a process by which epithelial cells gain migratory/invasive properties by upregulating pro-mesenchymal pathways, such as the expression of certain cadherin family proteins. Although a normal process during embryogenesis, EMT is often used by tumoral cells to increase their malignancy. In glioblastoma (GBM), a progressive glial tumor characterized by rapid growth and diffusely infiltrative spread, residing populations of glioma stem cells (GSCs) are thought to contribute to tumor invasiveness, EMT, and therapeutic resistance. To better understand GBM invasiveness in a developmental context, we used a recently described methodology to prospectively isolate human stem cell populations from GBM and germinal matrix tissues, and employed epigenetics to study their underlying transcriptional drivers. Analysis of RNA-seq in acutely-isolated GSC (vs. non-GSC) GBM populations revealed an upregulated EMT signature, with loss of "epithelial" markers, such as *DSP* and claudins (*CLDN1, 7, 11, 12*), and gain of mesenchymal markers, such as *TNC* and N-cadherin (*CDH2*). Chromatin accessibility mapping via ATAC-seq defined a tumor-specific signature related to migration, in which the TEAD1/4 transcription factor motif was the most highly and uniquely overrepresented in GSCs. Comparative analysis of ATAC-seq and RNA-seq data revealed several putative targets of TEAD1, and in vivo chromatin immunoprecipitation confirmed direct TEAD1 binding at the mesenchymal R-cadherin *CDH4*. Genetic ablation of TEAD1 in patient-derived GBM cells, using CRISPR-Cas9, reversed EMT transcriptome signature, diminished migratory properties in vitro, which were partially rescued after TEAD1 overexpression, and reduced profoundly infiltrative tumor burden in vivo after orthotopic xenotransplantation. CDH11 was one of the most downregulated mesenchymal cadherins after TEAD1 ablation, and its overexpression partially restored deficits in TEAD1-knockout cells related to cell migration, cell-cell adhesion, and substrate anchoring. Overall, our data validates a role for TEAD1 in GBM migration, mediated partially through the upregulation of EMT-associated cadherins.

ANGI-06. THE MATRIX PROTEIN THROMBOSPONDIN-1 IS A DOWNSTREAM TARGET OF TGF-β INDUCED MICROTUBE FORMATION IN GLIOBLASTOMA

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Microtubes (MTs), which are cytoplasmic extensions of glioma cells, have recently been discovered as important cell communication structures. MTs are abundant in chemoresistant 1p/19q non-codeleted tumors, in particular glioblastomas, however are scarce in chemosensitive 1p/19q co-deleted oligodendrogliomas. Here we report that TGF-β is an important mediator of MT formation. TCGA data analysis revealed upregulation of TGF-β growth-factors and receptors in non-codeleted versus co-deleted tumors. TGF-β stimulation in vitro promotes enhanced MT formation in a panel of GBM stem cell lines which was blocked by a TGFBR2 inhibitor (Ly2157299). Analysis of RNA sequencing data comparing TGF-β stimulated versus unstimulated cells revealed extracellular matrix receptor interactions as a major regulated pathway. We identified Thrombospondin-1 (THBS1) as a major candidate of this pathway, which was upregulated upon TGF-β stimulation in GBM stem cell lines. Interestingly, one GBM stem cell line that did not respond to TGF-β stimulation with enhanced MT formation lacked also upregulation of THBS1. This non-responder cell line did not invade into fetal microbrains in vitro and xenografts in vivo, whereas responder cell lines showed a highly invasive and MT forming phenotype in both models. Knockdown of THBS1 in a responder cell line using shRNAs substantially reduced MT formation in vitro and in vivo. Thus, we identified THBS1 as an important mediator of MT formation downstream of TGF-β, which might play a role in therapy resistance of GBM.

ANGI-07. 5ALA FLUORESCENCE BASED SORTING IDENTIFIES SERPINE1 AS A NOVEL THERAPEUTIC TARGET ON INVASIVE GBM CELLS

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INTRODUCTION: We previously described separating invasive tumor cells from within normal brain using 5ALA based fluorescent activated cell sorting (FACS). Using RNAseq on these cells we identify new potential therapeutic targets whose expression is elevated in tumor cells invading brain parenchyma. METHOD: 5-aminolevulinic acid (5-ALA) is a clinically used drug for fluorescence guided resection of GBM. Tumors from 11 patients were dissociated and FACS used to separate invasive fluorescent cancer cells from within normal brain. FACS sorted and unsorted mixed samples from tumor core, rim and invasive margin were compared. Gene expression was analyzed by RNA-seq and validated by qPCR, IHC and in vivo xenografts. RESULTS: Differential expression analysis identified 2567 genes with differences between core and invasive margin, and 78 genes with differences between 5-ALA FACS positive and FACS negative. Interestingly SERPINE1 expression is reduced in the unsorted invasive margin but expression remains high within sorted invasive tumor cells. Pathway analysis identified a predominance of immune system pathway changes between core and invasive margin. The differential expression of SERPINE1, VEGF, CHI3L1 and RTN1 in qPCR and IHC validated the same changes observed from the RNA-seq data. The invasive and tumorigenic capacity of 5-ALA positive sorted tumor cells was confirmed by enhanced engraftment in a mouse flank model compared to unsorted cells, whereas 5-ALA negative sorted cells failed to engraft. SERPINE1 knockdown had no effect on GBM cell proliferation but significantly reduced the ability of GBM cells to invade in an in vitro assay. CONCLUSION: This study has demonstrated that 5-ALA fluorescent sorting of the invasive region can identify new targets such as SERPINE1, whose high expression in invasive tumor cells would otherwise be overlooked. Our approach gives hope that we can interrogate true residual disease, identifying more relevant therapeutic targets.

ANGI-08. TARGETING THE RhoGEF BETA-PIX TO ENHANCE THE ACTIVITY OF BEVACIZUMAB IN GLIOBLASTOMA: A NANOPARTICLE MEDIATED GENE SILENCING APPROACH

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Glioblastoma (GBM), a highly invasive brain malignancy, remains an incurable disease. Angiogenesis, the formation of new vasculature, is a defining feature of this disease. Targeting GBM angiogenesis with Bevacizumab (Bev) is associated with improved progression free survival, but may also enhance tumour invasion into the surrounding parenchyma (Norden et al., 2008) and is not curative. Rho GTPases and their activators, guanine nucleotide exchange factors (GEFs), play central roles in the invasive process (4). Herein, we sought to identify and target GEFs of importance in mediating GBM invasion with a view to improving Bev response. We report a novel mechanism by which GBM tumours invade and proliferate *via* overexpression of the GEF beta-PIX gene which was shown to be increased at the invasive edge in 74% of GBM tumours assessed (n=19), compared with tumour core (Hoelzinger et al., 2005). We have further demonstrated that siRNA-mediated knockdown of beta-PIX in GBM patient-derived xenograft cell cultures and cell lines resulted in decreased cell invasion in 3D, cell proliferation and survival assays in vitro. An *in vivo* pilot study whereby beta-Pix knockdown was achieved using commercially available alphaV-beta3 integrin targeting nanoparticles (InVivoPlex Aparna Bio Corp), suggested that treatment with beta-PIX siRNA nanoparticles in combination with Bev could improve survival compared with Bev- alone in tumour-bearing animals. To further

develop this strategy, we have recently designed and characterized a proprietary novel biodegradable, RGD-targeting and cholesterol-stabilized polyplex system for siRNA delivery in GBM. This novel nanoparticle system supports efficient gene silencing, and demonstrates a low toxicity profile *in vitro* and *in vivo*. We are currently performing advanced pre-clinical efficacy studies employing a clinically relevant GBM rodent resection model (Sweeney et al., 2014), to determine if nanoparticle mediated beta-PIX gene silencing will improve survival outcomes when combined with Bevacizumb and delivered in the adjuvant setting.

ANGI-09. CIRCULATING MIR-10B AND MIR-21 IN PATIENTS WITH GBM TREATED WITH BEVACIZUMAB ARE SECRETED TO THE CIRCULATION BY PROTEINS AND THEIR HIGH QUANTIFICATION IS PROBABLY THE RESULT OF DRUG-INDUCED TUMOR HYPOXIA

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Bevacizumab is a one of the salvage therapy to patients with recurrent GBM. Antiangiogenic treatment such bevacizumab reduce blood supply and consequently increase hypoxia in the tumor microenvironment. We previously demonstrated that miR-10b and miR-21 are highly quantified in the circulation following bevacizumab treatment, and that the quantity of these miRNAs in the circulation is correlated negatively and significantly with tumor volume. miRNAs are selectively exported from tumors due to cellular signals or environmental cues and are selectively released to differential carrier such as exosomes, microvesicles, HDL or protein-associated miRNAs. The aim of the current study was to explore whether the upregulation of these miRNAs during the antiangiogenic treatment is induced by hypoxia, and via which carrier they are transported to the circulation. Three glioma and one endothelial cell line were exposed to hypoxic condition and the expression of VEGF and mir-10b and mir-21 were studied by quantitative-PCR. The cell medium as well as serum of patients treated with bevacizumab was fractionated by size-Chromatography-column (Izon's qEV) and then the quantification of the miRNAs in each fraction was measured by quantitative-PCR. Hypoxia induction was confirmed by upregulation of VEGF in both endothelial and glioma cell-lines. mir-10b and mir-21 were upregulated in the glioma cells and the glioma medium culture after 24Hr of hypoxia, but not in the endothelial cell-line Moreover, these miRNAs were enriched in the size fraction of protein and not in the other fractions. The results of this study imply that the high quantification of mir-10b and mir-21 in the serum of patients treated with bevacizumab are induced by tumor hypoxia and that these miRNAs are selectively secreted to the circulation by proteins. Further study is needed to investigate whether these circulating miRNAs and their delivery mode have a role in treatment response of GBM to bevasizumab.

ANGI-10. WSD1227: A BRAIN PENETRABLE VEGFR2 INHIBITOR FOR THE TREATMENT OF PRIMARY AND METASTATIC BRAIN TUMORS

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The VEGF pathway has emerged as an important target for cancer therapy by blocking the development of malignant neovasculature, thus to reduce oxygen availability to the tumor and decrease its growth. Anti-VEGF agents have been approved for several malignancies, such as GBM, NSCLC, mBC, CRC, OC, etc with satisfactory performance to extracranial lesions, but not in intracranial lesions. Insufficient penetration across BBB is one of factors limiting intracranial anti-tumor activity for those anti-VEGF agents, due to either large molecule weight or being substrate of BBB efflux transporters. The incidence of BM is increasing, and surgery/radiotherapy are the most common options for the management of BM. Patients with BM have a very poor prognosis and short survival. To address the role of anti-angiogenesis in the treatment of BM, a BBB penetrable, selective and potent VEGFR2 inhibitor WSD1227 is discovered with IC_{50} against VEGFR1/2/3 at 0.69/0.35/0.41nM versus against other targets such as $PDGFR_{\alpha}$ IC_{50} 22.9nM, $PDGFR_{\beta}$ IC_{50} 19.4nM, cKit IC_{50} 383nM, FLT3 IC_{50} 555 nM and CSF1R IC_{50} 1062nM. *In-vitro* MDCKII transfected cell assays demonstrated WSD1227 is not a substrate of P-gp or BCRP, two main efflux transporters on human BBB. Preclinical CNS PK studies confirmed brain penetration of WSD1227 with $K_{p,uu,brain}$ close to unity, thus not exacerbating VEGF related systemic toxicities. WSD1227 possessed superior PK profile with sufficient free PK exposure to achieve target engagement in mice. Treatment of tumor bearing mice in GBM, NSCLC, CRC and OC xenograft models with WSD1227 resulted in significant tumor growth inhibition. Predicted human PK properties are very promising to offer sufficient target engagement in clinic. Taken together, our data provide a good rationale for WSD1227 to be developed toward clinic to investigate anti-angiogenetic therapies for management of patients with primary or metastatic brain tumors.

ANGI-11. TUMOR TREATING FIELDS (TTFIELDS) INHIBIT CANCER CELL MIGRATION AND INVASION BY INDUCING REORGANIZATION OF THE ACTIN CYTOSKELETON AND FORMATION OF CELL ADHESIONS

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TTFields have been demonstrated to disrupt cancer cell replication in cultures, inhibit tumor progression in animals and increase overall survival in GBM patients when combined with temozolomide. TTFields are delivered via continuous, noninvasive application of low intensity, intermediate frequency alternating electric fields. In this study, we investigated the effects of TTFields on cancer cell migration and invasion properties. Glioma, breast, colon, melanoma and NSCLC cancer cell lines were treated with TTFields using the inovitro and the inovitro live systems. Cell migration rates were measured using wound healing assays. Invasion assays were performed using a modified Matrigel coated Boyden chamber. Cell adhesion assays were performed during TTFields treatment and compared to untreated controls. At the end of TTFields' treatment, adhered cells were trypsinized and counted. A cell de-adhesion assay was performed following different durations of TTFields application with the outcome being the number of cells removed after varying times of trypsinization. Confocal fluorescence microscopy imaging of vinculin and F-actin were utilized to demonstrate changes in cellular focal adhesions and stress fibers respectively following TTFields application. Application of TTFields in-vitro led to a significant reduction in the velocity of cell migration compared with untreated control cells. Cancer cell invasion was significantly reduced compared to untreated cells in all tested cell lines. In addition, cell de-adherence following TTFields treatment took significantly longer time of trypsinization. TTFields application also resulted in an increase in focal adhesion size and number as well as peripheral distribution of the adhesion sites. TTFields treated cells also adopted a more flattened and spread shape and exhibited reduction in appearance of stress fibers and a dense meshwork of actin filaments around the entire cell periphery. While typically regarded as an anti-mitotic treatment modality, TTFields may warrant further investigation and development for their anti-invasive and anti-metastatic potential.

ANGI-12. MRI-BASED CELL TRACKING WITH INDIVIDUAL CELL SENSITIVITY FOR MEASURING CANCER CELL INVASION

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PURPOSE: GBM cell invasion is particularly aggressive and leads to poor prognosis. *In vitro* and *in vivo* cell tracking techniques for studying tumor progression could facilitate drug discovery. Here we demonstrate the utility of MRI-based cell tracking as a tool for *in vitro* testing of inhibitors for cancer cell invasion. Iron oxide particles are the core technology that enables single-cell tracking, as labeled cells can be visualized as hypo-intensive spots using MRI. The technique provides a robust method to easily visualize cell invasion in 3D and can be applied *in vivo* to track invasion of labelled cells within the brain. METHODS: We developed an *in vitro* MRI assay capable of detecting invasion inhibition for human (U87MG) and rodent (RG2) glioma cell lines. We first established magnetic labelling efficiency (microscopy and ICP-OES) and cell viability/proliferation (MTT assay and PKH26 labelling) to determine optimal labelling concentration. Next, to visualize tumor cell invasion, magnetically labelled cells were formed into spheroids and embedded into a collagen matrix; samples were then scanned with a 7T MRI in a custom 3D printed holder. Cell invasion was visualized with and without inhibitor (CEP-1347, 400nM) and compared to traditional microscopy. RESULTS: Two scan sequences, T_1 FISP and FLASH, effectively visualized our 3D invasion assay; producing 3D images that demonstrated suppression of tumor cell invasion with inhibitor, equivalent to those observed with 2D microscopy. Further, cell viability and proliferation were not significantly impacted at 72hrs below bead concentrations of 0.1ug/mL. Tumor spheroids size was impacted by concentration ($F=4.820$, $p=0.001$)but not for cells labelled at the optimal 25pg/cell concentration. CONCLUSION: MRI can be used as a tool to monitor GBM invasion *in vitro* and can effectively test inhibitors for cancer invasion. This technique provides a powerful tool that can be used to observe invasion *in vitro* and *in vivo*.

ANGI-13. TENASCIN-C INDUCES VASCULOGENIC MIMICRY FORMATION IN GLIOBLASTOMA THROUGH AKT PATHWAY

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Glioblastoma is a highly invasive and vascularized primary CNS tumor. Although anti-angiogenesis therapy was effective in some tumors but not in GBM. Vasculogenic mimicry (VM), a vessel-like structure formed by highly invasive tumor cells, has been considered responsible for the failure of GBM patients for anti-angiogenesis therapy. Tenascin-c (TNC), an extracellular protein involves in tumor angiogenesis, is overexpressed in GBM. However, how TNC contributes to VM formation remains unclear. We first knocked down the expression of TNC in two glioma cell lines (U251 and A172), which had TNC overexpression. Then the formations of VM were observed by three-dimensional culture. Transwell and wound-healing assay were applied to investigate the role of TNC in cell invasion and migration abilities. The expression of MMP2, MMP9 and the phosphorylation status of AKT were determined by western-blot, RT-qPCR and gelatin zymography. Furthermore, AKT inhibitor, MK-2206 was used to block the AKT/MMP2/MMP9 signaling pathway to investigate its role in TNC regulation at different concentration (0μm, 5μm, 10μm and 20μm) for 24 hours. The knockdown efficiency was detected by western blot and RT-qPCR in U251 and A172 after siRNA transfection for 48 hours. Three-dimensional culture showed that VM-like structure formation decreased in knockdown groups compared to controls dramatically (19.67 ± 6.65 and 46.34 ± 9.10 vs. 125.67 ± 8.34 $p<0.01$ in U251; 59.67 ± 18.52 and 51 ± 14.97 vs. 100.67 ± 2.87, $p<0.01$ in A172). The invasion and migration ability of U251 and A172 were also attenuated ($p<0.01$) by TNC knockdown. TNC knockdown impaired the phosphorylation of AKT at both Ser473 and Thr308, and the expression of MMP2 and MMP9 were downregulated too. Exposure of AKT inhibitor blocked the VM formation as well as the expression of MMP2 and MMP9. Our study demonstrated that TNC upregulated the VM formation via AKT/MMP2/MMP9 pathway in glioma and provided a potential therapeutic target for glioblastoma by anti-angiogenesis therapy.

ANGI-14. EPIGENETIC REACTIVATION OF BAI1 SUPPRESSES TUMOR INVASION BY PREVENTING TGFβ1-INDUCED MESENCHYMAL SWITCH IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and lethal type of malignant brain tumor in adults. GBM cells are highly invasive and diffusely infiltrate throughout the brain, which strongly restricts multimodal therapies. Acquiring a better knowledge of molecular defects underlying GBM invasion is essential for the development of effective therapies. Brain-specific Angiogenesis Inhibitor 1 (BAI1) is a transmembrane receptor of the adhesion GPCR family widely expressed in normal brain, but its expression is lost in the majority of human brain tumors. We have previously shown that BAI1 is epigenetically silenced in most GBM and restoration of its expression can inhibit glioma growth in vitro and in vivo (Zhu D. *et al*, Cancer Res, 2012). Recently, we reported that BAI1 protects p53 from Mdm2-mediated degradation and regulate tumor growth in medulloblastoma (Zhu D. *et al*, Cancer Cell, 2018). However, it is unclear whether BAI1 loss is important for tumor invasion in GBM. We found that restoration of BAI1 expression in GBM cells suppressed mesenchymal transition. Microarray analysis of the TCGA dataset revealed that BAI1 expression inversely correlates with the expression of many key mesenchymal genes, including Fibronectin1, SLUG, and TWIST1. Reduced BAI1 expression also correlates with poor outcome. Restoration of BAI1 expression suppresses mesenchymal gene expression and dramatically decreases GBM cell brain invasion in mice. Mechanistically, the N-terminal thrombospondin type 1 repeat (TSR#1) of BAI1 inhibits the maturation process of TGFβ1, a key growth factor involved in EMT. BAI1 is silenced epigenetically in GBM cells by MBD2, and its expression can be reactivated by KCC-07, a blood-brain barrier permeable MBD2 inhibitor. We found that GBM cells treated by KCC-07 exhibited decreased tumor invasion. These experiments demonstrate that epigenetic silencing of BAI1 is important for activation of the GBM invasive phenotype through TGFβ1 pathway activation. Epigenetic targeting of this process by KCC-07 can reduce GBM invasion.

ANGI-15. PDGF-MEDIATED MESENCHYMAL TRANSFORMATION RENDERS ENDOTHELIAL RESISTANCE TO ANTI-VEGF TREATMENT IN GLIOBLASTOMA

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Angiogenesis is a hallmark of cancer. However, most malignant solid tumors exhibit robust resistance to current anti-angiogenic therapies that primarily target VEGF pathways. As such, bevacizumab, a humanized anti-VEGF monoclonal antibody, fails to prolong the overall survival time in patients with newly diagnosed glioblastoma (GBM). Here, we show that endothelial mesenchymal transformation induces GBM resistance to anti-VEGF therapy by downregulating VEGFR-2 expression in tumor-associated endothelial cells (ECs). Analyzing human GBM tumor-derived ECs and mouse EC lineage-derived cells in a genetically engineered GBM model, we show that VEGFR-2 expression is markedly reduced in GBM-associated ECs. Transcriptome analysis by RNA-seq shows abrogated VEGFR2 expression in GBM ECs and reveals a robust genetic reprograming with increased mesenchymal gene expression in these ECs. Furthermore, we identify a PDGF/NF-κB/Snail axis that controls mesenchymal transformation and reduces VEGFR2 expression, which induces EC resistance to anti-VEGF treatment. Finally, dual inhibition of VEGFR and PDGFR eliminates tumor-associated ECs and improves animal survival in the genetic mouse GBM model. Notably, EC-specific knockout of PDGF receptor-β (PDGFR-β) sensitizes tumors to antibody-based VEGF-neutralizing treatment. Taken together, these findings reveal a previously unidentified mechanism for anti-angiogenic treatment resistance, namely, cell plasticity-driven endothelial unresponsiveness to anti-VEGF treatment, and suggest that vascular de-transformation may offer promising opportunities for anti-vascular therapy in GBM and possibly other malignant solid tumors.

ANGI-16. EARLY DETECTION OF TUMOR CELL PROLIFERATION IS ASSOCIATED WITH A UNIQUE RADIOMIC BIOMARKER IN PRECLINICAL GLIOBLASTOMA XENOGRAFT AND PATIENTS

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PURPOSE: The mainstay imaging technique in brain tumor is Magnetic resonance imaging (MRI). However, early detection of tumor cell proliferation using MRI is limited due to inapparent disruption of normal brain architecture. Radiomics and machine learning techniques can quantitate thousands of imaging features that can depict neoplastic changes in apparently normal brain. Herein, we investigate the potential role radiomics can play in early detection of tumor cell proliferation in apparently normal MRI using a preclinically trained radiomic. METHODS: Two glioblastoma stem-like cell lines were transformed to stably express luciferase under a constitutive promoter. A stereotactic injection of tumor cells was performed to generate orthotopic mouse models (N=48). Tumor cell engraftment and *in-vivo* proliferation were assessed using bio-luminescence imaging (BLI) along with a weekly MRI (Bruker 7T). Images were analyzed, and ROIs were placed using 3D slicer software and radiomic features were extracted using Matlab. ROIs (0.75 mm) were placed on tumor injection sites and normal appearing contralateral brain. Radiomic features were compared for their significant alterations over time using comparative marker selection (CMS). Genomics and Histopathology of tumors were performed ex-vivo. Validation was performed in a cohort of brain cancer patients. RESULTS: Three stages of post-implantation tumor cell presence and proliferation were identified: 1. Immediate post implantation lag/engraftment phase. 2. Linear cellular proliferation phase (normal on conventional MRI). 3. Exponential cellular proliferation phase (apparent tumor on conventional MRI). Our data showed that 43% of extracted radiomic features were significantly changing (P Conclusion: Radiomic texture analysis and machine learning detects tumor cell presence and proliferation in normal-appearing brain prior to tumor development on conventional imaging. CLINICAL RELEVANCE: Radiomics and machine learning algorithms are predictive of tumor presence in seemingly normal MRIs. Early detection of tumors can allow earlier intervention, more extensive radiation planning and appropriately dose chemotherapeutic regimens.

ANGI-17. INTERLEUKIN 1 SIGNALING REGULATES BLOOD-BRAIN BARRIER INTEGRITY IN GLIOBLASTOMA

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Glioblastoma is the most common and aggressive primary brain tumor characterized by a dismal prognosis despite aggressive treatment regimens. A hallmark of glioblastoma is aberrant angiogenesis that results in disruption of the blood-brain barrier. This drives glioblastoma-associated cerebral edema caused by leakage of serum from the blood into the brain. Typically, edema is managed by the corticosteroid dexamethasone which is known to normalize the blood brain barrier. Various groups have also demonstrated beneficial effects following normalization of the blood-brain barrier with anti-angiogenic therapies such as cediranib and bevacizumab. Interleukin 1 (IL-1) signaling inhibition, however, has not been investigated in this con-

text despite evidence that it modulates blood-brain barrier integrity in other neuroinflammatory conditions. In this work, we demonstrate that IL-1 signaling does not impact the production of VEGF by bone marrow-derived macrophages and microglia *in vitro*. Moreover, we demonstrate no effect of dexamethasone treatment on VEGF levels in murine PDGFB-driven glioblastoma generated with RCAS/tv-a technology *in vivo*. This suggests that the restoration of blood-brain barrier integrity by dexamethasone is VEGF-independent. Dexamethasone is shown here, however, to downregulate both IL-1α and IL-1β expression *in vivo* and *in vitro*. Genetic ablation of IL-1β and subsequent MRI analysis is shown to reduce formation of edema *in vivo*, allowing mice to survive with larger tumors than wildtype controls. Serial sectioning of the tumor, and histological reconstruction of the total volume post-euthanasia confirms these results. Genetic ablation of IL-1R1 *in vivo* is shown to reduce blood-brain barrier permeability in a Hoechst dye-based vessel leakage assay. Future experiments will elucidate if these effects can be attributed to altered angiogenesis or regulation of endothelial cell junction molecules. Regardless, this work outlines IL-1 signaling as a promising therapeutic target to modulate blood-brain barrier integrity and serve as an alternative to dexamethasone for the treatment of glioblastoma-associated cerebral edema.

ANGI-18. AUTOCRINE CYCLE OF BONE MORPHOGENETIC PROTEIN 4 (BMP4) ENHANCES TUMOR AGGRESSIVENESS IN IDH1-MUTATED GLIOMAS

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BACKGROUND: *IDH1* mutations are the most prevalent genetic abnormality in WHO Grade II/III glioma. *IDH1*-mutated glioma exhibits distinctive tumor biology, including oncogenesis, invasiveness and therapeutic sensitivity. BMP4, a member of TGFβ superfamily, has been reported to promote tumor progression and invasiveness in various types of malignancies. In the present study, we investigated BMP4 signaling in *IDH1*-mutated glioma, seeking for the underlying molecular mechanisms as well as possible therapeutic approaches by targeting BMP4 pathway. METHODS: We analyzed the expression level of the BMP family in transcriptomic profiles of *IDH1*-mutated glioma. BMP4 expression was further quantified in clinical samples and patient-derived brain tumor initiating cells (BTIC). Further, we investigated the autocrine cycle of BMP4 to *IDH1*-mutated cells, focusing on the aggressive phenotype and cellular invasiveness. Finally, we analyzed the therapeutic value of BMP pathway inhibitors. RESULTS: Transcriptomic profiling showed significant up-regulation of BMP in *IDH1*-mutated glioma as compared with wild-type counterparts (8.20-fold for *BMP2*, 1.73-fold for *BMP4*). The up-regulation of BMP was confirmed in clinical samples through IHC. Moreover, we confirmed the up-regulation and secretion of BMP in *IDH1*-mutated BTIC. Autocrine of BMP not only activates concomitant Smad signaling, but also prompts cellular migration/invasion. Pharmacologic targeting BMP receptors by LDN-193189 suppressed Smad phosphorylation and cellular invasion. RNA sequencing and xenograft studies are currently ongoing to explore the molecular mechanism and therapeutic value of targeting BMP/Smad pathway for *IDH1*-mutated malignancies. CONCLUSION: Our preliminary findings showed that the BMP4 autocrine loop promotes the aggressiveness of *IDH1*-mutated glioma via Smad1 signaling. LDN-193189, the BMP receptor inhibitor, could be a potential therapeutic strategy to inhibit the aggressiveness of *IDH1*-mutated gliomas.

CELL BIOLOGY AND METABOLISM

CBMT-01. PARAOXONASE-2 IS HIGHLY EXPRESSED IN GBM AND PROMOTES GBM CELL SURVIVAL

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Paraoxonase-2 (PON2), a member of paraoxonase family, is an antioxidant enzyme that is implicated in cell survival. Recent evidence demonstrated that PON2 promotes apoptosis resistance in the selective tumor cells. Here, we report genetic regulation and role of PON2 in glioma. We showed that PON2 is highly expressed in glioblastomas (GBM) compared to non-tumor brains and that high PON2 expression is associated with poor survival in patients with GBM. Functional studies showed that knockdown of PON2 inhibits cell growth, induces apoptosis, and increases pro-apoptotic factor CHOP. Using transcriptome and transcription factor motif analysis, we showed that Nuclear Factor I-A (NFIA), a glioma-promoting transcription factor, positively regulates PON2 transcription and protein level and that PON2 and NFIA expression is highly correlated in GBM. Moreover, NFIA directly regulated PON2 transcription through binding to the PON2 promoter. Consistent with this finding, PON2 knockdown-induced growth inhibition is reversed by ectopic expression of NFIA. Furthermore, Nfia-deficient brain shows decrease in expression of PON2, providing genetic evidence that supports NFIA-PON2 regulatory relationship. Collectively, these data suggest that the pro-survival effect of PON2 is at least partly mediated by NFIA in GBM, advancing our understanding of the emerging role of PON2 in glioma.

CBMT-02. miR-124, -128, AND -137 COMBINATION THERAPY AGAINST GLIOBLASTOMA

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miRNAs are critical regulators of tumorigenesis, acting as oncogenes or tumor suppressors. Previous work, by us and others, have demonstrated that miR-124, -128, and -137 are key players in controlling neurogenesis and gliomagenesis. Together these miRNAs promote differentiation of neural stem cells (NSCs), while in gliomas their dysregulation contribute to the glioma phenotype. The three miRNAs display strong expression correlation in GBM cohorts and synergize to drive neuronal differentiation of NSCs by regulating a shared network of genes. Individually ectopic overexpression of these miRNAs induces significant changes in proliferation, and differentiation of GBM cells; however, it is unclear if they synergize to produce a stronger effect as observed in NSCs. We tested the combination of miR-124, -128, and -137 in glioblastoma cell lines and found that together they produce a synergistic effect, decreasing cell proliferation and we will test this synergy in other systems. In NSCs we found an interconnected transcription factor (TF) network to be important targets of miR-124, -128, and -137. Due to their importance in NSCs, we hypothesized that these TFs are critical in gliomas. We performed a siRNA screen against the TFs network in GBM cell lines and examined the impact of knockdown on cell proliferation, viability, and apoptosis. While several genes were important in maintaining the glioma phenotype, knockdown of ETS-related transcription factor Elf-4 (ELF4) produced the strongest phenotype changes. We will next characterize ELF4's transcriptional influence utilizing ChIP-seq paired with RNA-seq. Overall our initial data suggests that this miRNA-Transcription factor network is important in the glioma phenotype, and that combination of the three miRNAs is synergistic. Furthermore, we find the ELF4 is a critical TF from this network and regulates the glioma phenotype.

CBMT-03. A NOVEL METABOLIC PET TRACER STRATEGY TO DETERMINE EARLY EFFECTS OF TUMOR TREATING FIELDS (TTFIELDS)

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Pyruvate kinase M2 (PKM2) is a key marker of cancer metabolic reprogramming since it catalyzes the final step in glycolysis. [18F]DASA-23 is a novel radiotracer that measures aberrantly-expressed PKM2 in glioblastoma (GBM). Tumor treating fields (TTFields), the application of alternating electric fields (100–300 kHz, 1–4 V/cm) to tumors, is emerging as the fourth therapeutic modality in GBM after surgery, temozolomide (TMZ) chemotherapy, and radiotherapy. There is an important need to assess early on whether a patient's GBM is responding to a given therapy. We evaluated the ability of [18F]DASA-23 to detect changes in GBM metabolism in response to standard-of-care (TMZ) and emerging (TTFields) therapies. Human U87 GBM cells were subjected to 200 kHz TTFields, the IC_{50} of TMZ, or vehicle for three or six days (n≥3/condition), followed by evaluation of [18F]DASA-23 uptake. Immunofluorescence for PKM2 was performed to confirm the [18F]DASA-23 uptake results. Finally, Western blot analysis was performed to determine the effect of TMZ and TTFields exposure on the expression of PKM2 and a downstream enzyme, lactate dehydrogenase (LDH). 2-way ANOVA with multiple comparisons was performed. Data are reported as mean±SD. There was a significant interaction between the treatment (TTFields, TMZ, or vehicle) and treatment duration (3 or 6 days) on PKM2 expression as measured by [18F] DASA-23 uptake ($p=0.005$) at 30 minutes post-addition of radioactivity. Immunofluorescence independently confirmed reduced PKM2 expression due to TTFields. Western blot analysis revealed reduced PKM2 and LDH (normalized to loading control) without/with TMZ (0.43 ± 0.13 vs. 0.30 ± 0.05 [$p=0.14$] and 0.61 ± 0.16 vs. 0.43 ± 0.04 [$p=0.12$], respectively) and without/with TTFields (0.70 ± 0.23 vs. 0.15 ± 0.09 [$p=0.01$] and 1.14 ± 0.36 vs. 0.54 ± 0.14 [$p=0.04$], respectively). These data highlight the potential for non-invasive assessment of GBM's glycolytic response to standard and emerging therapies using [18F]DASA-23. A clinical study is underway to evaluate the ability of [18F]DASA-23 to predict responders vs. non-responders to anti-GBM therapy.

CBMT-04. INDUCING MITOCHONDRIAL OXIDATIVE STRESS AND TARGETING CELLULAR STRESS RESPONSE BY INHIBITION OF NAMPT, THE RATE LIMITING ENZYME IN THE NAD+ SALVAGE PATHWAY IN GLIOMA

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BACKGROUND: Pathways that regulate differential energy metabolism in gliomas have recently emerged as promising therapeutic targets. Nicotinamide Phosphoribosyltransferase (NAMPT), the rate-limiting enzyme of the NAD+ salvage pathway regulates key metabolic processes preferentially used in glioma energy metabolism and essential for tumor cell biology and proliferation. This study examines the effects of NAMPT inhibition using KPT9274 and FK866 (NAMPTi) on cellular respiration, oxidative stress and cytotoxicity to better delineate the role and regulation of NAD salvage pathway in gliomas. METHODS: Effects of NAMPTi on glycolysis and mitochondrial stress in gliomas and glioma stem cells (GSC) with varying IDH and MGMT status were measured including their oxidative state, basal cell respiration rate, maximum respiration capacity, spare respiratory capacity and proton leak using Agilent-Seahorse assay. Untargeted metabolomics was performed to analyze the effect of NAMPi on glioma cell metabolism. Effect of mitochondria dysfunction on cytotoxicity was measured by annexin-PI and CaspaseGlo assays. RESULTS: NAMPT inhibition caused NAD depletion, reduced ATP levels and PAK4 downregulation in gliomas. NAMPTi-treated cells showed reduction in basal cell respiration, spare and maximum respiration capacity indicating mitochondrial dysfunction and oxidative stress in glioma cells resulting in programed cell death. Untargeted metabolomics study indicated specific metabolic changes including accumulation of oxidized glutathione in NAMPTi-treated cells, indicating oxidative stress. Further, accumulation of metabolites indicative of a glycolytic pathway block and shunt towards the *de novo* purine synthesis pathway was seen. Further, glycolysis inhibition by NAMPTi was confirmed by glycolysis stress assays. Lastly, glycolysis inhibition and mitostress appeared to be related to a decrease in NAD+ dependent SIRT1 expression. CONCLUSIONS: NAMPTi cause profound disruption of mitochondrial function, induce oxidative stress and trigger cytotoxicity in GSC irrespective of MGMT promoter-methylation or IDH1 status. Targeting NAMPT is hence a novel therapeutic strategy that potentially circumvents tumor heterogeneity and can specifically disables tumor cell metabolism.

CBMT-05. ROLE OF THE let7-eEF2K AXIS IN MYC-DRIVEN MEDULLOBLASTOMA ADAPTATION TO NUTRIENT DEPRIVATION

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BACKGROUND/OBJECTIVES: *MYC* amplification in medulloblastoma (MB) determines highly aggressive disease, underscoring an urgent need for novel therapies. *Let-7* microRNAs (miRNAs) inhibit tumor progression and regulate metabolism by degrading several mRNAs, including MYC. *Let-7* miRNAs are frequently repressed in cancer, including MYC-driven MB. We previously reported that eukaryotic Elongation Factor-2 Kinase (eEF2K) is a pivotal regulator of MYC-driven tumor adaptation to nutrient deprivation (ND). Our data indicate that the eEF2K 3' untranslated region (UTR) harbors a potential binding site for *let-7*. In addition, eEF2K mRNA and *let-7* miRNA expression negatively correlates in MB, suggesting regulation of the former by the latter. We therefore hypothesized that let-7 down-regulation induces eEF2K expression in MB, thereby supporting MYC-driven MB adaptation to ND and tumor progression. METHODS: Immunohistochemistry for eEF2K substrate (p-eEF2) was performed on MB tissue microarrays to link results with MYC expression and clinical outcome. Effects of eEF2K pharmacological inhibition on MB cell survival were evaluated *in vitro* by MTT assays. The ability of *let-7* to degrade eEF2K mRNA was assessed by *let-7* miRNAs transfection into MB cells, followed by RT-PCR and Western Blotting for eEF2K. Binding of *let-7* to the eEF2K 3'UTR was validated by luciferase reporter assays. RESULTS: High eEF2K activity is linked to MYC over-expression and reduced survival in MB ($p<0.05$). Pharmacological inhibition of eEF2K significantly reduces survival of *MYC*-amplified MB cell lines under ND. Transfection *let-7* miRNAs decreases eEF2K mRNA and protein levels (by ~40–50%) in MB cells. Down-regulation of luciferase activity by *let-7* miRNAs is impaired upon mutation of the let-7 binding site on the eEF2K 3'UTR. CONCLUSIONS: *Let-7* miRNAs degrade eEF2K mRNA, indicating that *let-7* repression in MYC-driven MB is partially responsible for eEF2K increased levels and activity. Moreover, the *let-7*-eEF2K axis represents a critical mechanism for MYC-driven MB adaptation to ND, constituting a promising therapeutic target.

CBMT-06. LOWER GRADE ISOCITRATE DEHYDROGENASE (IDH) MUTANT GLIOMAS METABOLICALLY MIMICKING GLIOBLASTOMA (GBM) EXPRESS HIGHER R:S 2-HYDROXYGLUTARATE RATIOS RELATIVE TO NON-GBM-MIMICKING IDH MUTANT GLIOMAS

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BACKGROUND: Glioma patients continue to carry a very poor prognosis despite maximal therapy, urging for the development of novel therapies. Previous studies have identified mutations of metabolic enzymes directly regulating cellular metabolism, specifically, *isocitrate dehydrogenase* (*IDH1/2*) mutation. *IDH1/2* mutations occur early in glioma pathogenesis and result in the accumulation of oncometabolite 2-hydroxylgutarate (2-HG), with the preferential accumulation of the R relative to the S enantiomer of 2-HG. This metabolic reprogramming may help explain treatment resistance and highlight potential metabolic pathways involved in tumorigenesis. METHODS: To investigate the metabolic profile of a cohort of gliomas, we used ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) to detect a set of 689 metabolites in 90 *IDH1* mutant and *IDH1* wildtype gliomas. RESULTS: Unsupervised consensus clustering identified two distinct metabolic subgroups of gliomas: metabolic group 1, which consists of only *IDH1* mutated tumors (n=30) and metabolic group 2, which consists of all *IDH1* wildtype tumors and a subset of *IDH1* mutated tumors (n=60). We therefore, termed these *IDH1* mutated tumors in metabolic group 2 as "GBM mimickers". Importantly, comparison of *IDH* mutant tumors from metabolic group 1 versus the GBM mimickers showed distinct R:S 2-HG ratio differences, specifically with the GBM mimickers expressing significantly higher R:S 2-HG ratios (mean R:S 2-HG 2421.44 vs. 625.46, $p=0.014$). Moreover, the GBM mimickers showed significantly decreased progression free survival (20 months vs. 49 months, $p<0.05$, respectively) and increased tumor enhancement on imaging versus their *IDH1* mutated counterparts in metabolic group 1 (100% vs. 37.5%, $p<0.0001$), suggesting that R:S 2-HG ratio may be a marker of poor outcome. CONCLUSION: Our data supports distinct metabolic subtypes of *IDH* mutant gliomas, and highlights the use of R:S 2-HG ratio as a potential marker of poor outcome in *IDH* mutant gliomas.

CBMT-07. EVALUATION OF GLYCOLYTIC RESPONSE TO SEVEN CLASSES OF ANTI-GLIOBLASTOMA DRUGS BY NON-INVASIVE MEASUREMENT OF PYRUVATE KINASE M2

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INTRODUCTION: Pyruvate kinase M2 (PKM2) catalyzes the final and rate-limiting step in glycolysis, a key step in tumor growth and metabolism. PKM2 is preferentially expressed by glioblastoma (GBM) cells with minimal expression in the healthy brain, making it an important marker of cancer glycolytic re-programming. Our laboratory has developed 1-((2-fluoro-6-[18F]fluorophenyl)sulfonyl)-4-((4-methoxyphenyl)sulfonyl) piperazine ([18F]DASA-23), a novel radiotracer to noninvasively measure the expression of PKM2 and are currently conducting first-in-human studies with positron emission tomography (PET) imaging. METHODS: In this study we evaluated the ability of [18F]DASA-23 to detect GBM metabolic changes in response to seven classes of anti-GBM chemotherapy (n=11 drugs total). Human U87 GBM cells were subjected to the IC_{50} of alkylating agents (temozolomide [TMZ], carmustine, lomustine), a topoisomerase I inhibitor (irinotecan), inhibitors of vascular endothelial and epidermal growth factor receptors (cediranib and erlotinib, respectively) an anti-metabolite (5-fluorouracil), an anti-microtubule agent (vincristine), and metabolic therapies (AG-120 and dichloroacetate). After three or six days of exposure (n≥5/condition), the cellular uptake of [18F]DASA-23 was evaluated and PKM2 protein expression determined. PKM2 protein expression was compared via correlation analysis between two methods,

[18F]DASA-23 uptake and Western blot. Two-way ANOVA was used with corrections for multiple comparisons. Results are reported as mean±SD. RESULTS: There was a significant interaction between the interventions (n=13 including vehicle, media-only, and 11 drugs) and treatment duration (3 or 6 days) on PKM2 expression as measured by [18F]DASA-23 uptake (p=0.0001). The most significant change in [18F]DASA-23 uptake at XX minutes was evident in response to treatment with alkylating agents (p=0.0003), irinotecan (p=0.0055), erlotinib (p=0.04), and 5-fluorouracil (p=0.02). Western blot analysis revealed a moderate correlation between PKM2 protein expression [18F]DASA-23 uptake (R^2=0.44, p=0.15, Pearson correlation). CONCLUSIONS: These studies highlight the potential for evaluation of PKM2 expression with [18F]DASA-23 as a means to non-invasively monitor response to multiple classes of anti-GBM agents.

CBMT-08. COMPARISON OF THREE METABOLIC PET RADIOTRACERS IN GLIOBLASTOMA: CELL CULTURE AND ANIMAL STUDIES

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INTRODUCTION: Positron emission tomography (PET) is a non-invasive imaging modality used to visualize and define tumors based on their molecular characteristics and may play an important role in diagnosis and response assessment in glioblastoma patients. We have reported [18F]DASA-23 as a novel radiotracer for the measurement of aberrantly-expressed pyruvate kinase M2 (PKM2) in glioblastoma. PKM2 represents an attractive target for PET imaging due to lack of expression in the healthy brain and key role in the glycolytic reprogramming of glioblastoma. METHODS: We compared the uptake dynamics and in vivo performance of [18F]DASA-23 to that of established radiotracers ([18F]FDG and [18F]FDOPA). Human U87 glioblastoma cells were studied for the uptake experiments and were also orthotopically implanted into nude mice (N=4) for in vivo microPET/CT dynamic imaging with the three radiotracers. Finally, a separate cohort of mice (N=6) was evaluated with [18F]DASA-23 microPET/CT before and one week post-initiation of vehicle or temozolomide chemotherapy (70 mg/kg, oral, 5 days per 28-day cycle). Data are reported as mean±SD. RESULTS: The cellular uptake at 60 minutes post-addition of radioactivity was 10.5 ± 0.5 %uptake/mg protein for [18F]DASA-23, which was significantly greater than that of [18F]FDG (1.94 ± 0.1, p<0.0001) and [18F]FDOPA (1.2 ± 0.1, p<0.0001). In the animal imaging studies at 30 minutes post-injection of radioactivity, [18F]DASA-23 clearly delineated the tumors from the surrounding healthy brain tissue and had a significantly higher tumor-to-brain ratio compared to that of [18F]FDG and [18F]FDOPA (4.1 ± 0.4 vs. 1.2 ± 0.2 [p=0.0005] and 2.6 ± 0.2 [p<0.0001], respectively). In another cohort of mice, the percent change in the [18F]DASA-23 tumor-to-brain ratio from pre-treatment to one week post-treatment was significantly reduced in the temozolomide compared to the vehicle group (p=0.004). CONCLUSIONS: These studies highlight the benefit of PET imaging with [18F]DASA-23 over other radiotracers ([18F]FDG and [18F]FDOPA) and suggest the potential to detect early response to temozolomide therapy with [18F]DASA-23.

CBMT-09. ESTABLISHMENT AND IN VITRO CHARACTERIZATION OF A SPORADIC PEDIATRIC ATYPICAL MENINGIOMA CELL LINE

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Meningiomas represent ~30% of primary neoplasms in adults but only 0.4–4.6% in children. Due to the paucity of cases, relatively few studies have addressed pediatric meningiomas and there are insufficient cell lines to conduct *in vitro* studies. We have established and characterized a new cell line (PED-24) from a 12-year-old male who presented with headaches and subsequently underwent gross total resection of a sporadic, large left frontal meningioma, WHO grade 2. The tumor was focally positive for EMA and GFAP. Whole-exome sequencing of the patient was negative for NF-2. Compared to three high grade glioma lines (two H3K27M DIPG tumors and one adult GBM), PED-24 showed significantly increased growth kinetics as assessed by real-time live-cell analysis. The cells were also successfully cultured as neurospheres in neurobasal medium. Ultrastructural analysis with SEM and TEM demonstrated unique cellular morphology. Average cellular diameter was 3.2 ± 0.3 µm and the cells had a microvilli-covered smooth surface. There was a high burden of transport vesicles and an extensive network of endoplasmic reticulum with evidence of autophagy events. This line provides a resource for further exploration of pathogenic mechanisms involved in sporadic pediatric meningiomas. One novel avenue of interest is the potential presence of a stem-like subpopulation within sporadic pediatric atypical meningiomas.

CBMT-10. GLUTAMINE DEPRIVATION ALTERS ONE-CARBON METABOLISM TO MAINTAIN GLIOMA CELL SURVIVAL

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Cancer cells acquire and utilize necessary nutrients to survive from a frequently poorly nutrient environment. This metabolic processes include cellular biosynthesis, redox maintenance and epigenetic regulation through nucleic acid and protein methylation, leading to selection for cancer cells with increasing tumorigenicity and therapy-resistance. But less is known about how cancer cells alter metabolism to support cell growth and survival from nutrient starvation. Here, we identify that one-carbon metabolism integrated cellular nutrient status by cycling carbon units from amino acids to support cell proliferation. To identify metabolic response to glutamine deprivation in glioma cells, we analyzed metabolites using gas chromatography and mass spectroscopy (GC/MS) in glioma cells cultured in glutamine-deprived medium and examined gene expression of key enzymes for one-carbon units using RT-PCR and western blotting methods. These expressions were also confirmed by immunohistochemical staining in glioma clinical samples. Metabolome studies indicated serine, cysteine, and methionine as key differentiating amino acids between control and glutamine-deprived groups. Gene expression analysis identified upregulation of Phosphoserine aminotransferase 1 (*PSAT1*)Serine hydroxymethyl transferase 2 (*SHMT2*), and Methylenetetrahydrofolate dehydrogenase 2 (*MTHFD2*) to regulate serine synthesis and one-carbon metabolism. Importantly, suppression of these metabolites impaired glioma cells in glutamine-deprived condition. In human glioma samples. *SHMT2* and *MTHFD2* expressions were highest in poorly nutrient regions around "pseudopalisading necrosis". One-carbon metabolism has a key role for glioma cells to survive glutamine deprivation. These results may suggest the new therapeutic strategies targeting critical glioma cells adapting the tumor microenvironment.

CBMT-11. PEROXISOMAL FATTY ACID OXIDATION IN GLIOBLASTOMA DURING HYPOXIA

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BACKGROUND: Peroxisomal fatty acid oxidation (FAO) offers some unique metabolic advantages compared to mitochondrial FAO that may be important to cancer cells adapting to chronic hypoxia exacerbated by anti-angiogenic treatment. When peroxisomal FAO is dominant, the resulting low $FADH_2$/NADH ratio allows for more efficient oxygen utilization and lower oxidative stress production during oxidative phosphorylation. Our previous findings suggest cells from tumors treated with bevacizumab are more sensitive to peroxisomal FAO inhibition. The current study further examines changes in peroxisomes, mitochondria, and their FAO pathways, and their roles in adaptation to hypoxia and anti-angiogenic treatment. METHODS: Peroxisomal number was determined by electron microscopy. Gene expression was determined by QT-PCR. RESULTS: Metabolomic analysis by NMR/MRS revealed glioblastoma cells under chronic hypoxia (2–5 weeks) likely utilize less mitochondrial FAO and showed signs of increased oxidative stress. In some cell lines we observed that chronic hypoxic cells were more sensitive to treatment with a peroxisomal FAO inhibitor, particularly in an anti-angiogenic resistant tumor cell line. Increased expression (RQ>2) of the genes ACADVL, ACADS, and DECR2, suggests an increase in mitochondrial FAO for very long and short fatty acids and increase in peroxisomal FAO for unsaturated fatty acids. There were higher overall levels of peroxisomes in the anti-angiogenic resistant tumor cell line and lower levels of mitochondria in hypoxia compared to normoxia for cell lines which were sensitive to peroxisomal FAO inhibition (p<.05). CONCLUSIONS: Both chronic hypoxia in vitro and through anti-angiogenic treatment appear to produce changes in peroxisome and mitochondria levels and FAO pathways which we are currently further characterizing with additional cell lines and animal experiments.

CBMT-12. FATTY ACID SYNTHASE POSITIVE EVs AS NOVEL BIOMARKERS IN BRAIN CANCER.

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BACKGROUND: Extracellular vesicles (EVs) are known for their important role in cancer progression and hold considerable potential as tumor

biomarkers. However, purification of tumor-specific EVs from plasma is still a pressing need since contamination by normal host cell-EVs, results in compromised analytical sensitivity. Fatty acid synthase (FASN) was recently shown by us to be present on EVs from cultured glioma cells. Here we analyzed circulating patient EVs for the expression of FASN, CD9, CD81 and CD63. METHODS: Plasma EVs from patients with glioblastoma (n=24), anaplastic astrocytoma (n=6) and healthy controls (n=16) as well as EVs from early passage glioma stem cells (GSCs) were analyzed for FASN, CD9, CD81, CD63 by imaging flow cytometry (IFC). EVs were further investigated by nanoparticle tracking analysis (NTA), electron microscopy and immunoblotting. Glioblastomas, anaplastic astrocytomas and normal brain tissue specimens were analyzed for FASN by Western blotting and immunohistochemistry. RESULTS: FASN is elevated in glioblastoma tissue compared to noncancerous brain and FASN expression levels correlate with WHO grade (p+/CD81$^+$and FASN$^+$/CD63$^+$EVs are present in plasma from patients with glioblastoma and anaplastic astrocytoma as determined by IFC (p+EVs and double positive (CD9$^+$/CD63$^+$, CD9$^+$/CD81$^+$or CD63$^+$/CD81$^+$) EVs (p CONCLUSION: FASN expression is elevated on circulating EVs from patients with malignant gliomas and FASN is a promising marker for the identification and enrichment of glioma-derived plasma EVs, an important prerequisite for in-depth genetic, epigenetic and transcriptional analyses that could inform clinicians on molecular alterations and help monitoring treatment efficacy.

CBMT-13. UNRAVELLING METABOLISM OF GLIOBLASTOMA USING MASS SPECTROMETRY IMAGING

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INTRODUCTION: Glioblastoma Multiforme (GBM) is the most frequent malignant brain tumour in adults. The dismally low survival rates require novel diagnostic and therapeutic approaches. Understanding the tumour metabolic landscape in relation to its microenvironment is paramount. To address this issue, we have used high-resolution Mass Spectrometry Imaging (MSI). METHODS: A female wistar rat implanted intracranially with C6-GBM cells was infused with [U-^{13}C]glucose (a bolus of 0.4 mg/g body weight followed by infusion with 0.012 mg/g/min at 300 µL/h for 2 h) at 15 days post cell implantation. The presence of tumour at this point was confirmed in a T_2-weighted magnetic resonance image. Post-infusion, the rat brain was rapidly excised and fixed by rapid freezing in liquid nitrogen. Coronal cryosections were subjected to desorption electrospray ionization (DESI) MSI. The mass-to-charge (*m/z*) ratios of ^{13}C-labelled and unlabelled metabolites were compared between different brain regions using unsupervised spatial clustering. Naïve rat brain (not infused with [U-^{13}C]glucose) was used as a control for natural abundance ^{13}C. RESULTS: An increase in circulating blood glucose levels from 8 to 9.5 mmol/L was observed at the end of [U-^{13}C]glucose infusion. Unsupervised clustering of metabolite *m/z* ratios distinguished different regions of the brain and normal brain from tumour. The profile of metabolite *m/z* ratios also showed intra-tumoural metabolite heterogeneity with differences between tumour boundary and core regions. CONCLUSIONS: These results demonstrate that C6-GBMs are metabolically heterogeneous and different from surrounding brain tissue. MSI can thus help unravel spatial differences in tumour metabolism. Comparison of MSI data to histological staining of brain sections can yield additional information about the relationship between the tumour and its microenvironment.

CBMT-14. EXPLOITING VULNERABILITIES OF THE MESENCHYMAL SUBTYPE OF GLIOMA STEM-LIKE CELLS TO ENDOPLASMIC RETICULUM STRESS

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Transcriptome profiling of glioma stem-like cells (GSCs) show differences in biological properties depending on their expression signature. Mesenchymal (MES) GSCs are generally associated with increased treatment resistance, thus it is important to identify essential molecules that promote their survival. Here, we discover the preferentially higher expression of the endoplasmic reticulum (ER) chaperone protein glucose-regulated protein 78 (GRP78) in the MES subtype of glioblastomas (GBMs) and derivative GSCs. MES GSCs showed lesser activated basal unfolded protein response (UPR) compared to its proneural (PN) counterparts. GRP78 knockdown in GSCs increased the basal level of UPR and enhanced tunicamycin induced apoptosis. GSCs expressing high levels of GRP78 concomitantly possess higher content of protein and lipid droplets (LDs). As shown by metabolomics studies we have performed in MES GSCs, silencing GRP78 stimulated biosynthesis of lipids that we hypothesize might protect MES GSCs from ER stress induced reactivation oxygen species (ROS) accumulation and apoptosis. In fact, concomitant treatment with simvastatin, a cholesterol inhibitor, induced apoptosis in cells with silenced GRP78 *in vitro* and significantly improved survival *in vivo*. Our study shows that higher GRP78 expression and lipid biosynthesis in MES GSC might act as protective mechanisms from ER and oxidative stress, which can be exploited for therapeutic targeting of the MES subtype of GSCs.

CBMT-15. METABOLIC AND TRANSCRIPTIONAL PROFILES OF GBM INVASION: COMPARISON OF PATIENTS AND PAIRED PATIENT DERIVED XENOGRAFTS USING^{1}H MAGNETIC RESONANCE SPECTROSCOPY AND IMAGING (7T AND 14T) AND RNA-SEQUENCING

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BACKGROUND: Glioblastoma (GBM) are notorious for their invasive behavior. Little is known about the biology of the tumor cells and the microenvironment at the invasive front that is highly relevant for recurrence. These cells are technically difficult to visualize, lie usually outside the resected and irradiated area, and are behind the blood brain barrier that renders them difficult to treat. We present patient specific metabolic and transcriptomic features of invasive growth using corresponding patient derived xenografts (PDX). METHODS: Patients with suspected GBM were enrolled and underwent ^{1}H magnetic resonance spectroscopy and imaging (^{1}H-MRS/I) at 7 Tesla. Tissue obtained at the subsequent resection was dissociated and transplanted orthotopically into mice (n=4 to 6). Mice were followed longitudinally by ^{1}H-MRS/I (14T). The PDX, the corresponding tissue of the contralateral side, and the original tumors were subjected to RNA sequencing. Tumor versus host (mouse) derived sequencing reads are computationally separated. RESULTS: Diffuse xenografts developed for 8 (IDHwt) of 9 patients within 2–5 months. ^{1}H-MRS at ultrahigh fields allowed reliable quantification of 22 metabolites. The temporal changes of the metabolite signatures characterized the kinetics of invasive growth on both, the injected and the contralateral side. At end stage the signatures corresponded well with histological findings. Migration to the contralateral side ranged in a patient dependent manner between < 1 to > 50% 50% of tumor cells. Comparison of MRS derived metabolite signatures at end stage and the corresponding human signatures compared best with voxels measured outside the core of the human tumor. Associations between the metabolite signatures and the transcriptome in the xenografts and the host will be discussed. CONCLUSION: Integration of metabolic profiles and gene expression of the tumor and the invaded brain may provide insights and tools for *in vivo* monitoring of treatment response in the infiltration zone. GRANTS: Swiss Bridge Award, Swiss Cancer Ligue

CBMT-16. A COMBINATION STRATEGY TO COUNTERACT PTEN-DEFICIENCY BY TARGETING THE S6 AND TAM KINASES IN GLIOBLASTOMA

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PTEN deficiency affects the majority of glioblastomas, triggering signal transduction pathways that induce anabolic metabolism and apoptosis resistance. The mTOR complex 1 (mTORC1) and S6 kinases (S6Ks) are key mediators of metabolic reprogramming and apoptosis resistance in PTEN-deficient cells. S6K1 can be inhibited using LY2584702, a selective inhibitor that has been tested in Phase 1 clinical trials. We show here that LY2584702 can be used in combination with the TAM tyrosine kinase inhibitor BMS777607 to interfere with anabolic metabolism and restore cell death pathways in PTEN-deficient glioblastoma cells. In vivo, combination treatment reduced the growth rate of PTEN-deficient glioblastoma cells in both subcutaneous and orthotopic tumor settings. The data indicate that the TAM kinases, consisting of the tyrosine kinases TYRO3, AXL, and MERTK, can be targeted together with the S6Ks to counteract PTEN-deficiency in glioblastoma.

CBMT-17. NOVEL APPROACH OF UTILISING SERUM/PLASMA EV AND CELL-FREE RNA FOR TREATMENT MONITORING IN GLIOBLASTOMA PATIENTS

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INTRODUCTION: Glioblastoma (GBM) is a malignant primary brain tumour with dismal prognosis. Treatment monitoring remains a challenge in clinical routine, since brain imaging cannot reliably differentiate between true progression and treatment-associated changes. In this project, we evaluate different methods of extracellular vesicles (EV) purification, in order to specifically isolate GBM-EVs from human serum/plasma and introduce EVs, as well as cell-free RNA as possible biomarkers for treatment monitoring in GBM patients. METHODS: EVs from primary GBM cells and the Gaussia luciferase expressing Gli36-GLuc cells were isolated via size-exclusion chromatography (SEC) and ultracentrifugation. EV-surface markers were evaluated by flow cytometry. Gli36-GLuc EVs containing GLuc mRNA were spiked in healthy plasma. Thereafter, plasma EVs were isolated via ultracentrifugation, SEC and immunoprecipitation. Subsequently, RNA was isolated from vesicles and evaluated for GLuc levels via qRT-PCR. Total cell-free RNA from serum of GBM patients was tested for different mRNAs and micro-RNAs at different disease stages. RESULTS: EVs from GBM cells expressed high levels of CD29 and CD44, when compared to EVs from healthy donor plasma. Gli36-GLuc EVs spiked in healthy plasma were more effectively isolated with CD44-based immunoprecipitation than with ultracentrifugation or SEC, as shown by higher GLuc RNA levels in the corresponding vesicles. When compared to total cell-free RNA extracted from this plasma, RNA from EVs exhibited a higher GLuc yield. In cell-free RNA from GBM patients, MGMT levels alone were not capable of detecting progressive disease. CONCLUSIONS: 1. CD44 could serve as a novel, promising target for GBM-EV and be utilised for immunoprecipitation-based EV capturing. 2. Using the appropriate EV purification method possibly affects their potential as biomarkers for GBM. 3. MGMT levels alone in cell-free RNA of GBM patients did not correlate with disease status contrary to previous reports.

CBMT-18. INTEGRATIVE CROSS PLATFORM ANALYSES IDENTIFY ENHANCED HETEROTROPHY AS A METABOLIC HALLMARK IN GLIOBLASTOMA

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Although considerable progress has been made in understanding molecular alterations driving gliomagenesis, diverse metabolic programs contributing towards is aggressive phenotype remains unclear. We performed integrative cross platform analyses coupling global metabolomic profiling with genomics in patient-derived glioma (low-grade astrocytoma [LGA; n=28] and glioblastoma [GBM; n=80]) to define and provide molecular context to metabolic reprogramming driving gliomagenesis. Clear metabolic programs were identified differentiating LGA from GBM, with aberrant lipid, peptide and amino acid metabolism representing the most dominant metabolic nodes associated with malignant transformation. Although the metabolomic profiles of GBM and LGA appeared mutually exclusive, considerable metabolic heterogeneity was still observed in GBM. Surprisingly, these integrative analyses demonstrated that MGMT methylation and IDH mutation status, which represent two of the strongest prognostic factors in GBM, were equally distributed among GBM metabolic subtypes. Transcriptional subtypes, on the other hand, tightly clustered by their metabolomic signature, with proneural and mesenchymal tumors' profiles being mutually exclusive. Extending genomic signatures of individual metabolic phenotypes to Ivy GAP, we demonstrated the observed metabolic subtypes were a function of *intra-* rather than*inter*-tumoral heterogeneity. Integrating these metabolic phenotypes with gene expression analyses uncovered tightly orchestrated and highly redundant transcriptional programs designed to support the observed metabolic programs by actively importing these biochemical substrates from the microenvironment. These findings were metabolomically, genomically, and functionally recapitulated in preclinical models by demonstrating the potential of subtype-specific GBM lines to actively important fatty acids and protein/amino acids from the environment. This contributed to a state of enhanced metabolic heterotrophy supporting survival in diverse microenvironments that are implicit in this malignancy. Collectively, we demonstrate that despite disparate molecular pathways driving the progression of GBM, metabolic programs designed to maintain its aggressive phenotype remain conserved and are a function of its diverse tumor ecology.

CBMT-19. RNU6-1 ANALYSED IN EXOSOMES FROM SERA AS A NOVEL DIFFERENTIAL BIOMARKER FOR GBM VS NON-NEOPLASTIC BRAIN LESIONS AND NSCPL

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The identification of circulating biomarkers by non-invasive methods would be helpful for glioma detection and response assessment. Strong evidences have shown that GBM cells release microvesicles containing proteins and RNA. We have previously demonstrated that exosomes isolated from the serum of GBM patients had an increased expression of RNU6-1 compared to control samples; therefore it could serve as a non-invasive diagnostic biomarker for GBM. In this study, we set to investigate the role of RNU6-1 as a differential biomarker of GBM versus other brain diseases with similar radiological features. RNU6-1 expression was analysed by digital droplet PCR (ddPCR) in circulating exosomes from serum samples of GBM patients (n=18), healthy controls (n=28), and patients with different brain lesions: subacute stroke (n=30), acute-subacute haemorrhage (n=29), acute demyelinating lesions (n=19), brain metastases (n=21) and Primary CNS Lymphomas (PCNSL) (n=12). We observed that the expression of RNU6-1 was significantly higher in GBM patients (412 ± 550.48 copies/20µL) than in healthy controls (150 ± 224.35 copies/20µL; p=0.039) validating our preceding results. Furthermore, RNU6-1 levels were increased in exosomes from GBM patients than in exosomes from patients with non-neoplastic lesions (stroke [223 ± 709.8 copies/20µL; p=0,067], haemorrhage [127 ± 198.7 copies/20µL; p=0.010], demyelinating lesions [111.5 ± 250.35 copies/20µL; p=0.019]) and PCNSL [18.15 ± 245.7 copies/20µL; p=0.004]). Contrary, RNU6-1 levels were similar between brain metastases and GBM patients [325 ± 632 copies/20µL; p=0.573]. In addition, assessing RNU6-1 as a predictive marker of GBM by ROC curves analysis, we demonstrated that RNU6-1 was a robust diagnostic biomarker of GBM compared to subacute stroke [AUC=0.659; p=0.004], acute/subacute haemorrhage [AUC=0.724; p=0.006], acute demyelinating lesions [AUC=0.728; p=0.011] and PCNSL [AUC=0.814; p<0.001]; in contrast, it did not allow differentiating GBM from brain metastases [AUC=0.552; p=0.575]. Our data indicate that RNU6-1 from circulating exosomes could serve as a differential biomarker for GBM versus non-neoplastic brain lesions and PCNSL, therefore, be used as a non-invasive method for GBM diagnosis.

CBMT-20. A KETOGENIC PILL FOR GLIOBLASTOMA

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INTRODUCTION: While the KD holds promise as a therapeutic option for brain cancer patients, stringency of the diet impacts compliance. We have previously demonstrated that a high fat/low carbohydrate diet, similar to the Ketogenic Diet [KD], can reduce tumor progression and enhance survival in an orthotopic xenograft model. However, while this diet is less restrictive than the classic KD it still involves significant changes to a patient's diet. Two of the primary physiological changes that occur when on the KD are a reduction in glucose and an increase in ketone bodies. These physiological changes are mimicked by providing ketone esters [KE] in the diet, and we [AMP, DPD], have recently shown that ketone esters can reduce glucose, elevate ketone bodies and enhance survival in a metastatic cancer model. HYPOTHESIS: We hypothesize that KE [1,3 butanediol acetoacetate diester] will reduce glucose, elevate ketones, reduce tumor progression and enhance survival in an orthotopic xenograft GBM model using a PDX model. APPROACH: NON/SCID animals implanted with patient-derived GBM cells were fed a standard diet [SD], or SD + KE [20%] till they reached endpoint. Body weight, plasma glucose, and ketones were measured weekly and overall survival assessed. RESULTS: While the KE is bitter and can have poor compliance, we found that supplementing with 1% Stevia increased palatability based on food consumption and body weight. Comparing to SD, KE supplemented diet reduced plasma glucose (145.5 ± 5.3 vs 121.7 ± 5.7), increased ketone bodies [ß-hydroxybutarate, 0.7 ± 0.15 vs.1.3 ± 0.1] and enhanced median survival [47 ± 6.2 vs 60.8 ± 1.9, days]. CONCLUSION: Ketone esters can be effectively delivered orally together with a standard diet, and produce similar physiological changes [reduction in glucose and elevation in ketones] and enhance survival as the more restrictive KD in NON/SCID animals.

CBMT-21. ALTERATIONS OF CYSTEINE METABOLISM IN GENETIC VARIANTS OF HIGH GRADE GLIOMAS

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Isocitrate dehydrogenase (IDH) mutation have been reported to impose in gliomas a shortage of NADPH required to maintain a redox state and may rely on cysteine (Cys) availability for biosynthesis of glutathione (GSH) to ensure antioxidant levels. Cys may be replenished via extracellular intake or by de novo intracellular synthesis via transsulfuration (TS) pathway. The aim of this study was to investigate alterations of Cys metabolism in genetic variants of high-grade gliomas (HGG). Seventeen tumor samples from 15 adult patients (11 M / 4 F; average age 57 years, range 25 – 81 years), who underwent surgical resection for newly diagnosed or recurrent HGG were analyzed by HPLC. Levels of Cys, homocysteine and GSH were correlated with the genetic signature of HGG (wild-types vs. IDH1 mutation, PTEN deletion, EGFR amplification and MGMT methylation). Cys levels were significantly higher (2.1 fold increase; p=0.0038) in IDH1-mut (n=4) vs. IDH1-wt HGG (n=13), with comparable homocysteine and GSH levels. PTEN deletion and EGFR amplification did not significantly alter Cys metabolites with comparable levels of Cys, homocysteine and GSH detected in PTEN-del (n=7) and PTEN-intact (n=6) HGG, as well as in EGFR-amp (n=7) and EGFR-non amp (n=9) HGG. Significantly higher Cys levels (3.2 fold increase; p=0.0186) were also found in MGMT methylated (n=4) vs. non-methylated (n=3) HGG, with comparable levels of homocysteine and GSH. Increased Cys levels detected in IDH1-mut and MGMT methylated HGG support the hypothesis that these tumors may preferentially use the TS pathway for GSH synthesis. These findings are consistent with our report of increased TS pathway enzyme cystathionine B-synthase (CBS) in HGG, but concurrent increased intake of Cys cannot be excluded. Our results suggest utilizing Cys metabolites as potential markers and/or therapeutic targets in some genetic variants of HGG, a hypothesis that should be further explored in larger translational trials.

CBMT-22. The PI3K/mTOR PATHWAY CONTRIBUTES TO SEX DIFFERENCES IN GLIOBLASTOMA

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Glioblastoma (GBM) occurs more commonly in males but female GBM patients survive significantly longer. Therefore, understanding the molecular mechanisms that underlie these sex differences may be an innovative approach for developing novel treatments. We found that EGFR amplification and increased mTOR phosphorylation worsen outcome for men, but not women with GBM. To investigate the contribution of the PI3K/mTOR pathway to sex differences in GBM biology and outcome, we used a murine model of GBM with inactivation of Neurofibromin 1 and p53 function, which has previously yielded important insights into sexual dimorphism in GBM. We found that PI3K/mTOR pathway activity was significantly greater in male GBM cells upon treatment with the activating ligands EGF, insulin or IGF-1. Furthermore, when we treated GBM cells with the targeted mTORC1 inhibitor temsirolimus, male GBM cells were significantly more resistant to pathway inhibition, underlining our hypothesis that the PI3K/mTOR pathway is sexually dimorphic in GBM. One mechanism by which the PI3K/mTOR pathway regulates GBM cell growth is through the regulation of metabolic pathways. Furthermore, high expression levels of glycolytic genes worsens outcome in male glioma patients but not females, suggesting there are sex differences in glioma metabolism. To discover sex differences in PI3K/mTOR pathway-regulated metabolism, we performed a targeted metabolomic screen of male and female GBM cells treated either with insulin or vehicle. Multiple central carbon metabolites, involved in nucleotide metabolism, NAD metabolism, and TCA cycle, were significantly different in male and female cells. We further confirmed the validity of these findings with controlled nutrient deprivation and supplementation studies in cell culture and found that male GBM cells were more sensitive to those manipulations. Together, these data indicate that the PI3K/mTOR pathway and its metabolic targets are sexually dimorphic in GBM and that metabolic targeting approaches may be particularly effective in male GBM patients.

CBMT-23. MODULATION OF HYPERSYNAPTIC MICROENVIRONMENT DIFFERENTIALLY PROMOTES GLIOMAGENESIS ACROSS PIK3CA VARIANTS

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Genetic mutations remain one of the principle drivers for glioblastoma multiforme, a devastating disease with the poorest prognosis; however, much like other cancers mutations are both a cause and consequence of glioma. While there is a wealth of patient genomics data, they do not properly discern or address whether annotated genetic perturbations are causative "driver" events or consequential "passenger" mutations. To address this, we developed a screening platform where we can screen up to 50 different genetic factors, in vivo. In combination with next generation sequencing, we identified the most potent candidate drivers. Additionally, we've comparatively analyzed 30 different alleles of PIK3CA in vitro. Using these approaches, we identified candidate drivers and individually validated these alleles. Testing 7 variants in vivo, we identified C420R and M1043I as rare and previously uncharacterized driver mutations in GBM. Additionally, we identified variants that do not promote tumor growth, demonstrating that each variant differentially drives gliomagenesis. Subsequent molecular profiling of driver variants suggested differential synaptic environments which correlated with seizure activity. We further characterized this synaptic deregulation, revealing that individual variants can differentially modulate the hypersynaptic microenvironment, independent of tumor context. Taken together with recent work revealing that local synaptic activity can promote tumor growth, this would suggest that specific variants of PIK3CA differentially drive gliomagenesis by differentially modulating the hypersynaptic profile of the tumor microenvironment. In sum, our study reveals: 1) a novel screening approach whereby we can screen 50 different factors in vivo, 2) novel GBM-relevant driver alleles C420R and M1043I previously not characterized in vivo, 3) that even single amino acid alterations across driver mutations can drastically alter gliomagenic programs, and 4) one differential mechanism by which PIK3CA variants differentially promote gliomagenesis is by deregulating synaptic microenvironment.

CBMT-24. MITOCHONDRIAL DNA CONTENT IN GLIOBLASTOMA AND ITS CLINICAL SIGNIFICANCE

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Evidence of altered mitochondrial DNA (mtDNA) content in several malignancies has surfaced in recent years. However, in glioblastoma, this aspect remains obscure. Hence, we have studied mtDNA content in glioblastoma at diagnosis and recurrence. METHODS: Formalin fixed paraffin embedded tissue of newly diagnosed glioblastoma (n=100), paired recurrent glioblastoma (n=16 pairs) and non-neoplastic brain (n=30) archived in the department of Neuropathology, NIMHANS, were utilized for the study. Clinical details of the patients were obtained from files. IDH, ATRX and TERT promoter mutations, MGMT promoter methylation and EGFR amplification were assessed using immunohistochemistry, Sanger's sequencing, methylation specific PCR and fluorescent insitu hybridization. mtDNA copy number was analyzed using quantitative real time PCR (relative quantification). The mtDNA content was calculated as percentage of normal using the formula $2^{-\Delta\Delta CT}$ x 100. Hence, for example, 20% mtDNA copy number means that if non-neoplastic brain tissue contains 100 copies of mtDNA, the tumor contains only 20 copies. RESULTS: mtDNA content was lower than non-neoplastic brain tissue (mean mtDNA copy number 19.8%) in all cases. Among them, the mtDNA content was significantly lower in older patients (p=0.04). Tumors expressing markers of aggressiveness had lower mtDNA content, though the difference was not statistically significant. Survival analysis using Cox regression showed that lower mtDNA copy number is associated with higher risk and hence poorer prognosis (Exp(B):0.97; p=0.045). Of the16 patients with recurrence studied, 7 had received radiation therapy (RT) while the others defaulted or recurred before initiation of RT. mtDNA content had increased at recurrence(mean:48.02%) when compared to the primary tumor(mean:20.32%) in all 7 cases who received RT. In the remaining 9 cases who did not receive RT, the difference was not statistically significant. Thus, for the first time, we show that lower mtDNA copy number is associated with poorer survival and earlier recurrence in glioblastoma and that RT may increase the mtDNA content.

CBMT-25. ANTI-TUMOR EFFECT OF VEGF INHIBITOR PLUS KETOGENIC DIET THERAPY FOR GLIOBLASTOMA

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INTRODUCTION: Malignant glioma cells depend on glucose as the main energy source. Cancer cells may not be able to metabolize ketones as efficiently as normal brain cells, the ketogenic diet (KD) has been proposed as a complementary therapy for treatment of malignant gliomas. VEGF (vascular endothelial growth factor) inhibitor (bevacizumab) decreases blood supply to tumor and clinically used for glioblastoma treatment. Therefore, we examined anti-tumor effect of the combination of bevacizumab (Bev) and KD therapy using mouse model. METHODS: U87MG cells were implanted into the right brain of nude mice. One week after the implantation, mice were randomized into four treatment groups: control group, KD group, Bev group, and combination group. KetoCal 4:1 was administered to the mice for KD. Bev (10mg/kg) was injected from tail vein twice a week. Metabolic and histological analysis of the tumor, and survival analysis of the mice were performed. RESULTS: 3-hydroxy-butyrate, one of the ketone bodies, was increased in the tumor of KD group, and principal component analysis (PCA) analysis demonstrate distinct clustering or a clear separation of the four groups. Histologically, density of neovascularization was more increased in the combination group, compared with control, and phospho-ERK expression and Ki-67 index were decreased in the combination group. There was no different in body weight between KD group and other groups, and there was no different in survival time between KD group and control median overall survival 26 days vs 23 days, p=0.11. However, Bev group had significant longer survival than control group median OS 40 days, p=0.0016, and the combination group had most longer survival time among four groups median OS 50 days, p=0.0015. CONCLUSIONS: Drastic metabolic remodeling in the tumor occurred in the combination of Bev and KD. This combination may be potentially useful for glioblastoma therapy.

CBMT-26. HIGH MOBILITY GROUP AT-HOOK 2 (HMGA2) IS A PROGNOSTIC FACTOR ASSOCIATED WITH MALIGNANT PHENOTYPE IN MEDULLOBLASTOMAS

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Medulloblastoma is an embryonal tumor arising in the cerebellum and its molecular mechanism has been revealed to be classified in several groups. The constitutive expression of high mobility group AT-hook 2 (HMGA2) is associated with malignant phenotype and reduced survival in some cancers. However, the clinical and biological significances of HMGA2 in medulloblastoma have not been elucidated. Here, we examined the expression, prognostic and therapeutic role of HMGA2 in medulloblastoma. HMGA1 and HMGA2 expressions were examined in 20 patients with medulloblastoma using immunohistochemical study. High expression levels of HMGA1 and HMGA2 were frequently identified in medulloblastoma tissues. In addition, high expression of HMGA2 correlated with high proliferation marker Ki-67 labeling index. High expression of HMGA2 was significantly associated with progression free survival and overall survival of patients with medulloblastoma (p=0.021 and p=0.016, respectively). To examine the function of HMGA2 expression, knockdown HMGA2 expression was applied in Daoy, D283 and D341 cells. Knock-down of HMGA2 resulted in inhibited cell proliferation, migration/invasion and enhanced apoptosis in vitro. In conclusion, HMGA2 overexpression is frequent in medulloblastoma, and its expression is related to proliferation marker and poor prognosis. These findings suggest that HMGA2 could serve as a potential target for future therapeutic strategies in medulloblastoma.

CBMT-27. PROTEOMIC MAP OF GLIOMA BIOPSIES REVEALS FUNCTIONAL DEFECTS IN ENDOCYTOSIS

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BACKGROUND: Decades of molecular genetic analyses have shown that gliomas accumulate genetic alterations that result in the enhanced activity of growth factor receptor tyrosine kinases (RTKs) and mediators of downstream pathways. Among them are gain-of-function of *EGFR*, *PDGFR*, *PIK3CA*or *BRAF*, and loss-of-function of *PTEN* or *NF1*, resulting in exacerbated proliferative responses. METHODS: We performed deep proteomic analysis of human gliomas of distinct genetic backgrounds, *i.e. IDH*, *TERT* statuses in combination with *EGFR* or *PDGFRA* amplification. RESULTS: Mass spectrometric analysis confirmed the R132H mutation in the *IDH*-mutant biopsies. Proteomic quantification not only revealed strikingly high levels of EGFR protein in biopsies carrying EGFR amplification when compared to control biopsies from white matter, but also overexpression of EGFR irrespective of *EGFR* copy number status, genetic background, tumor histology or grade. Furthermore, proteomic data and Western blot analysis showed a general decrease in the expression of core components of clathrin-mediated endocytosis, like adaptin (AP2), clathrin (CLT) and dynamin (DNM) in tumors of all genotypes, histology or grades. Functional binding assays in two primary cell lines yielded identical binding of transferrin to its receptor, even though one contained much reduced transferrin receptor levels. This clearly shows that reduced endocytosis leads to prolonged residence time of receptors on the plasma membrane. CONCLUSION: These three groups of proteins are indispensable for clathrin-mediated endocytosis and inactivation of growth factor receptors. Thus, loss of endocytosis proteins increases the availability of RTKs at the plasma membrane by prolonging growth factor signaling, leading to a selective advantage in tumorigenesis and progression. We are currently performing electron microscopy to visualize impaired clathrin vesicle formation in glioma cell lines and extending our uptake assay to RTKs like EGFR. Further, we are currently investigating on a general endocytosis gene shut down in gliomas by epigenetic silencing.

CBMT-28. THE PROSURVIVAL ROLE OF HEME OXYGENASE-1 AND ITS REGULATION BY EGFRvIII IN GLIOBLASTOMA: SURGICAL AND PHOTODYNAMIC THERAPY APPLICATIONS

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BACKGROUND: The extent of surgical resection of Glioblastoma Multiforme can be greatly improved using 5-aminolevulinic acid (5-ALA) induced fluorescence-guided surgery. Yet, the intensity of the 5-ALA induced fluorescence is heterogeneous and may vary between patients. We have previously determined that Heme Oxygenase-1 is strongly involved in the metabolism of Protoporphyrin IX (PpIX), the fluorescent metabolite of 5-ALA (Fontana *et al*. 2017). Noteworthy, our experiments have shown that the EGF-mediated activation of Epidermal Growth Factor Receptor (EGFR) and its constitutively active truncation version, EGFRvIII is able to significantly increase the expression of HO-1 in glioblastoma cells. We are now investigating the role of Heme Oxygenase-1 in glioblastoma progression and possible future implications for 5-ALA-based photodynamic therapy. METHODS: We measured the effect of EGFR/EGFRvIII status on the downstream effect on Heme Oxygenase-1 (HO-1). 5-ALA induced fluorescence, cell proliferation and survival were measured by different techniques. Furthermore, effects of inhibition by Tin(IV)-Protoporphyrin (SnPP) or gene knockdown using small interfering RNA (siRNA) against *HO-1*were analyzed. RESULTS: A significant difference in fluorescence was observed in U87MG and U87EGFR (EGFR overexpression) compared to U87EGFRvIII (EGFRvIII overexpression). Treatment with EGF, but not TGF-α significantly reduced cellular fluorescence by promoting HO-1 transcription and expression in a concentration-dependent manner, and the effect was reversed by HO-1 inhibition. We show evidence that this effect is mediated by pAKT/NF-kβ pathway activation. Furthermore, inhibition of HO-1 activity was significantly associated with an increase in apoptosis and reduced cell proliferation and survival. CONCLUSION: In GBM cell lines, 5-ALA-induced fluorescence is variable, and modulated by EGFR/EGFRvIII pathway activity inducing HO-1. EGFRvIII was shown to induce higher levels of HO-1 and therefore reduced cellular fluorescence. Furthermore, HO-1 protein expression was associated with cell survival and treatment with HO-1 inhibitors may reverse its anti-apoptotic/pro-survival effect and be therefore a new tool in GBM treatment.

CBMT-29. INDUCTION OF AUTOPHAGY FOLLOWING TTFIELDS APPLICATION SERVES AS A SURVIVAL MECHANISM MEDIATED BY AMPK ACTIVATION

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Tumor treating fields (TTFields) are an approved treatment modality for patients with glioblastoma. Previous studies have shown that TTFields lead to increased cellular granularity, which is often associated with autophagy. In this study, we evaluated the effect of TTFields on the induction of autophagy in glioma cells. Cells were treated with TTFields using the inovitro system. Autophagy was monitored by quantifying levels of lipidated Microtubule Associated Protein Light Chain 3 (LC3-II) using immunoblotting and immunofluorescence microscopy. Transmission Electron Microscopy (TEM) was used to visualize autophagosome-like structures. Western blot analysis was

utilized to evaluate autophagy regulatory activity of mTOR through p70 S6 kinase1 and AMPK and its down-stream target ULK-1. siRNAs was used to deplete siAMPK from U87-MG cells. To evaluate involvement of autophagy in cell fate after TTFields treatment, we produced glioma cell lines depleted from ATG7 by shATG7 infection. Significant elevation in LC3-II levels was observed in TTFields-treated cells using fluorescence microscopy; punctate distribution of LC3-II was observed. TEM micrographs demonstrated the presence of autophagy typical, autophagosome-like structures following TTFields treatment. Evidence of increased autophagic flux following TTFields treatment was detected using immunoblotting analysis in the presence of CQ. Western blot analysis of cells after TTFields treatment revealed the stimulation of AMPK signaling as well as activation of p70. Depletion of AMPK from U87-MG cells resulted in reduction of autophagy and enhancement of TTFields cytotoxicity. ER stress in treated cells was evident by immunoblotting, showing increased levels of ER stress marker GRP78. The combination of TTFields with CQ resulted in a significant dose-dependent reduction of cell growth compared with TTFields treatment alone. Cells with ATG7 depletion showed similar results. Combined, these results suggest that cells upregulate autophagy in response to ER stress induced by TTFields application and that AMPK may serve as a key regulator of this process.

CBMT-30. ASSESSMENT OF PRECEDENCE RELATIONSHIPS BETWEEN IDH1 MUTATION AND GLS2 EXPRESSION ON 5-ALA FLUORESCENCE IN GLIOMAS

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Fluorescence-guided surgery with 5-aminolevulinic acid (5-ALA)is widely utilized modality for detection and resection of malignant gliomas. We have previously reported that the mutational status of IDH1 and the expression level of glutaminase 2(GLS2) are associated with the 5-ALA fluorescence in gliomas. This study is to investigate the dominant factor on 5-ALA fluorescence between IDH1 and GLS2 in gliomas. Primary cultured human glioma cells and malignant glioma cell lines were used to engineer the IDH1 mutational status and GLS2 expression. Functional analysis and in vitro experiments confirmed that GLS2 is the dominant factor over IDH1 mutation for intracellular protoporphyrin IX (PpIX) accumulation and fluorescence and in gliomas. NADPH production capacity was associated with in accordance with IDH1 mutational status and GLS2 expression level, and correlated with fluorescence. GLS2 underexpression was the dominant factor for the 5-ALA fluorescence in gliomas over IDH1 mutation.

CBMT-31. QUIESCENT GLIOBLASTOMA CELLS SHIFT TO AN EPITHELIAL-MESENCHYMAL TRANSITION (EMT)-LIKE GENE PROGRAM

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Glioblastoma (GBM) is the most common malignant primary brain tumor in adults, with a median survival rate of less than 15 months. Quiescent stem cells of GBM are potential sources for recurrence after therapy. Therapeutic targeting of this quiescent population in combination with therapies that target proliferative cells may be able to completely eradicate GBM tumors, however, our understanding of the physiology and molecular features of quiescent GBM cells is still limited. To isolate quiescent GBM stem cells and to investigate their molecular signature, we have engineered GBM cells with an inducible histone2B-GFP reporter (iH2B-GFP) by CRISPR/Cas9 knock-in. Quiescent GBM cells retain GFP-high label after pulse-chase paradigms. We utilized a 3D GBM organoid model that mimics tumor heterogeneity, including the formation of a quiescent population. GFP-high quiescent GBM cells were subjected to stem cell assays and RNA-Seq expression analysis. While quiescent GBM cells were similar in clonal culture assays to their proliferative counterparts, they displayed higher therapy resistance. RNA-Seq analysis of quiescent and proliferative GBM cells showed upregulation of EMT pathway genes and also induction of genes that modify the extracellular matrix. After exposure to irradiation or temozolomide, we observed increased fractions of the GFP-high population, demonstrating higher therapy resistance. Histological analyses of tumors growing in mouse host brains revealed the relative prevalence of quiescent cell populations near putative perivascular stem cell niches. Our findings connect quiescent GBM cells with an EMT-like shift, possibly explaining how GBM stem cells achieve high therapy resistance and invasiveness, and suggest new targets to abrogate the stem cell population of GBM. In addition, the upregulation of ECM components suggests that quiescent GBM cells may actively form their own extracellular "niche", in line with the concept that a distinct microenvironment determines the physiology of GBM stem cells.

CBMT-32. IMAGING A HALLMARK OF CANCER: TERT EXPRESSION LEADS TO MRS-DETECTABLE METABOLIC REPROGRAMMING

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Hotspot promoter mutations reactivate expression of telomerase reverse transcriptase (*TERT*) in mutant IDH1 oligodendrogliomas. TERT expression allows glioma cells to avoid telomere dysfunction-induced senescence, thereby enabling limitless proliferation. Since TERT expression is essential for glioma proliferation and TERT inhibitors are attractive therapeutic targets, identification of metabolic biomarkers of TERT expression will facilitate non-invasive imaging of tumor status and response to therapy. Therefore, the goal of this study was to identify ^{1}H- and hyperpolarized ^{13}C-magnetic resonance spectroscopy (MRS)-detectable metabolic biomarkers of TERT expression in mutant IDH1 glioma cells. We studied mutant IDH1-expressing immortalized normal human astrocytes without (NHA_{pre}) and with TERT expression (NHA_{post} and NHA_{tert}). Using ^{1}H-MRS we monitored steady-state metabolite levels and analyzed the data in an unbiased manner using principal component analysis. Our results indicated that the metabolic profile of NHA_{pre} cells differed significantly from that of NHA_{post} and NHA_{tert}. Elevated levels of glutathione (GSH, reduced), NADPH, NADH, aspartate and taurine in NHA_{post} and NHA_{tert} cells relative to NHA_{pre} were responsible for this discrimination. These results identify ^{1}H-MRS-detectable biomarkers of TERT expression in mutant IDH1 glioma cells. Next, we used ^{13}C-MRS to examine [2-^{13}C]-glucose flux to the pentose phosphate pathway (PPP) since the PPP is a major source of NADPH, which, in turn, maintains GSH levels. PPP fractional flux was significantly higher in NHA_{post} and NHA_{tert} cells relative to NHA_{pre}, consistent with higher levels of NADPH and GSH in the TERT-expressing models. Importantly, the flux of hyperpolarized [U-^{13}C]-glucose to the PPP intermediate 6-phosphogluconate was elevated in NHA_{post} and NHA_{tert} cells relative to NHA_{pre}, thereby identifying hyperpolarized [U-^{13}C]-glucose as a potential imaging biomarker of TERT status. Collectively, our study links TERT expression to significant metabolic reprogramming in mutant IDH1 gliomas and identifies ^{1}H- and hyperpolarized ^{13}C-MRS biomarkers that may be valuable for monitoring tumor TERT status and response to therapy.

CBMT-33. ALTERNATING ELECTRIC FIELDS INDUCED BY FAST SPINNING STRONG MAGNETS MODULATE MITOCHONDRIAL ENERGY METABOLISM IN GBM CELLS

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Recently, scalp application of alternating electric fields has shown therapeutic benefit in patients with glioblastoma multiforme (GBM). The U.S. Food and Drug Administration has approved this new "Tumor Treating Fields (TTF)" therapy provided by Novocure (trade name Optune) as monotherapy for recurrent GBM and in combination with temozolomide for newly diagnosed GBM. Two multicenter Phase III clinical trials demonstrate that in the former condition TTF treatment after standard of care chemotherapy only increases median survival from 6 to 6.6 months, and in the latter condition combination chemo-TTF therapy, compared to chemotherapy alone, prolongs progression free survival from 4 to 7.1 months. Although this treatment is highly promising, its underlying biophysical and molecular mechanisms are unclear. "Transcranial Rotating Permanent Magnet Stimulation" (TRPMS) is a similar approach in which non-invasive multifocal magnetic stimulation produces the required alternating electric fields by electromagnetic induction. Ongoing pilot TRPMS trials in chronic ischemic stroke and other neurological patients reveal a complete absence of significant TRPMS device-related adverse effects. Here, we report pre-clinical tests of the hypothesis that TRPMS-based alternating electric fields cause depolarization of the mitochondrial membrane potential (MMP) and alter mitochondrial oxidation of nutrients, leading to reactive oxygen species (ROS) and cytochrome C-mediated apoptosis in patient-derived GBM cells. Using ^{13}C NMR spectroscopybased isotopomer analysis in GBM cells we observe that after 3 hours of treatment with TRPMS-induced electric field stimulation there is an increased metabolic flux of glycolysis and decreased mitochondrial glucose oxidation in the TCA cycle. Fluorescence microscopy with MitoTracker demonstrates changes in staining intensity that are consistent with TRPMS-induced disruption of mitochondrial networks due to depolarization of MMP, processes that culminate in apoptosis triggered by release of ROS and cytochrome C. These findings, if supported by *in-vivo* data in mouse models, would suggest an important role of TRPMS in GBM treatment.

CBMT-34. TARGETING THE RNA-PROCESSING GENE MAGOH AS A NOVEL THERAPY FOR MEDULLOBLASTOMA

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We have proposed that microcephaly-producing mutations identify genes required for growth in the nervous system that may be new targets for brain tumor treatment. We tested this hypothesis by deleting the RNA-processing gene *Magoh* during cerebellar development and medulloblastoma pathogenesis. *Magoh* mutation has been shown to induce DNA damage and apoptosis in forebrain progenitors, resulting in microcephaly. We conditionally deleted *Magoh* during postnatal cerebellar neurogenesis by crossing $Magoh^{loxP/loxP}$ mice with CAG-CreER transgenic mice ($Magoh^{Cre\text{-}ER}$). *Magoh*-deleted cerebellar granule neuron progenitors (CGNPs) of the cerebellum of $Magoh^{Cre\text{-}ER}$ mice showed DNA damage, cell cycle arrest and apoptosis, which depleted the progenitor-rich external granule layer within 72 hrs. Moreover, deleting *Magoh* in SHH-driven medulloblastomas in SmoA1 mice similarly induced DNA damage, apoptosis and tumor shrinkage. These data show that MAGOH is required for SHH-driven proliferation in both normal cerebellar progenitors and in SHH-driven medulloblastoma. These findings are the first to show that medulloblastoma growth can be disrupted by targeting the RNA processing machinery.

CBMT-35. MicroRNA ANALYSIS OF THE INVASIVE MARGIN OF GLIOBLASTOMA REVEALS DRUGGABLE THERAPEUTIC TARGETS IN LIPID METABOLISM PATHWAYS

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BACKGROUND: Heterogeneity of gene expression in Glioblastoma (GBM) has been recently recognised as a key feature involved in therapy resistance with invasive cells remaining after surgery and displaying unique molecular features. The dysregulation of small non-coding RNAs known as microRNAs (miRNAs) can disrupt gene regulatory networks and contribute to GBM development. However, the intra-tumour heterogeneity of miRNA expression in GBM has not yet been investigated. MATERIAL AND METHODS: Global miRNA expression profiling was performed in 45 GBM surgical specimens sampled from the tumour necrotic core, proliferative rim and the invasive margin. Gain-of-function studies were conducted using transfection of selected mature miRNAs into GBM cell lines. Global metabolomic profiling by liquid chromatography coupled with electrospray mass spectrometry (LC-ESI-MS) and gene expression assays were used to identify molecular targets regulated by miRNAs in GBM cells. Transwell assays were performed to examine the migration and invasion effects of inhibiting targets expression. Evaluation of targets expression in tumour tissue samples was performed by qRT-PCR, flow cytometry and immunohistochemical analysis. RESULTS: We identified two significantly upregulated miRNAs in the invasive margin compared to the core and the rim of the tumour. Integrated metabolomic and gene expression analysis revealed that these miRNAs may act synergistically to target key enzymes involved in fatty acid oxidation (ACOX1 and CPT2) and lipoprotein uptake and secretion (LDLR). ACOX1, CPT2 and LDLR were found to be expressed at lower levels in the core and the rim relative to the tumour invasive margin. The pharmacological inhibition of CPT2 and LDLR resulted in a significant reduction in GBM cell invasion. CONCLUSION: Our finding indicates that lipid metabolism may present a possible vulnerability of GBM invasive margin. Understanding the function of miRNAs in regulating lipid homeostasis in GBM may provide novel avenues for GBM therapy.

CBMT-36. GRK2 PROMOTES MEDULLOBLASTOMA GROWTH AND SURVIVAL

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BACKGROUND: GRK2 (G-protein coupled receptor kinase 2) is a ubiquitous member of the GPCR family that phosphorylates agonist-stimulated GPCRs, leading to b-arrestin mediated receptor internalization and recycling. GRK2 can also phosphorylate non-GPCR substrates and participate in various signaling networks related to cell proliferation and survival. Despite the potential connectivity of GRK2 to signaling pathways related to cell proliferation and survival, there are few reports of GRK2 in cancer, and none in medulloblastoma. Medulloblastoma is the most common malignant brain tumor in children. Here we demonstrate for the first time that GRK2 has a pro-growth effect in medulloblastoma cell lines and that it mitigates cell death in response to the chemotherapeutic agent, cisplatin. METHODS: flow cytometry, SDS-PAGE, western blotting. CELL LINES: UW228 and Daoy medulloblastoma cell lines (SHH subgroup). RESULTS: 1. GRK2 knockdown inhibited cell proliferation and slowed growth rate of both medulloblastoma cell lines. 2. GRK2 overexpression increased cell proliferation and growth rate in both medulloblastoma cell lines. 3. GRK2 silencing increased basal and cisplatin-induced cell death in both medulloblastoma cell lines, whereas GRK2 upregulation mitigated this cisplatin effect. 4. GRK2 K220R (catalytically-inactive) and GRK2 S670A (cannot be phosphorylated) both mitigated the cisplatin-induced cell death to similar degree as wild type GRK2. Conversely, GRK2 S670D (phosphomimic) lost the ability to mitigate cisplatin-induced cell death, suggesting that GRK2 phosphorylation at S670 is inhibitory and blocks the pro-growth effect of GRK2 in these medulloblastoma cell lines. 5. Akt phosphorylation at serine 473 was diminished in Daoy cells with GRK2 knockdown compared to controls. CONCLUSIONS: Our data for the first time identify pro-growth and pro-survival roles for GRK2 in medulloblastoma cells and a modulatory effect of GRK2 on cisplatin-induced cell death of these medulloblastoma cells.

CBMT-37. FDA-APPROVED HDAC INHIBITORS ANTAGONIZE THE WARBURG EFFECT AND CAUSE UNIQUE METABOLIC VULNERABILITIES

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Histone deacetylase inhibition is a potential new strategy for the treatment of glioblastoma (GBM) and FDA-approved for multiple myeloma. However, resistance to therapy is a major issue. By conducting a global transcriptome with subsequent gene set enrichment analysis in stem-like GBM cells and PDX models coupled with a comprehensive polar and non-polar untargeted metabolite analysis, we have unraveled that HDAC – inhibition results in global reprogramming of metabolism, revealing unique metabolic vulnerabilities. Acute HDAC – inhibition suppresses c-myc protein levels and thereby blunts glycolysis and the pentose phosphate pathway. Acutely, this results in lower ATP levels coupled with activation of AMPK, as well as dysregulation of redox equivalents. Furthermore, GBM cells activate mitochondrial metabolism oxidative phosphorylation (OXPHOS) with an increase in mitochondrial fusion, mitochondrial biogenesis and higher mitochondrial oxygen consumption rate coupled with increased fatty acid oxidation (FAO), which is mediated by the master regulator transcription factors, PGC1a and PPARD. In turn, GBM cells become addicted to OXPHOS and susceptible to apoptosis induction by inhibitors of mitochondrial translation and OXPHOS. In like manner, the clinical validated CPT1 inhibitor, etomoxir, reduces oxygen consumption rate in HDAC – inhibitor treated cells and synergistically induces apoptosis in GBM cells, highlighting the dependence of HDAC – inhibitor treated cells on FAO. HDAC – inhibitor resistant cells display a highly mitochondrial oxidative phenotype with increased number and size of mitochondria and substantial higher oxygen consumption rate, a transcriptional signature of FAO and reliance on oxidative energy metabolism. To fuel FAO, HDAC – inhibitor treated cells activate lysosomal, autophagic signaling. Finally, we show that interference with mitochondrial energy metabolism along with HDAC – inhibition results in synergistic growth inhibition in six xenograft model systems, reemphasizing the translational relevance of our findings.

CBMT-38. ASSOCIATION OF miR-146aC>G, miR-149C>T, miR-196a2C>T, AND miR-499A>G POLYMORPHISMS WITH BRAIN TUMORS

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MicroRNAs (miRNAs) are short, noncoding RNAs that are implicated in tumorigenesis, functioning as both tumor suppressors and oncogenes. However, the clinical significance of miRNA expression profiles for brain tumors remains unclear. Therefore, we designed a study to investigate the association between microRNA genetic variants and brain tumor risk. We recruited 362 participants; 179 for the healthy subjects and 183 who were brain tumor patients confirmed as having gliomas, meningiomas, or schwannomas. This study investigated the single nucleotide polymorphisms *miR-146a*C>G, *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). We found that the dominant *miR-149* and CC genotypes were significantly more frequent in patients with glioma. The odds ratios for the C-C-C-G, C-T-C-G, and G-C-T-G haplotypes (*miR-146a*C>G-*miR-149*T>C-*miR-196a2*T>C-*miR-499*A>G) were sig-

nificantly increased in glioma, as was the odds ratio for the GCT haplotype of *miR-146a*C>G, *miR-149*T>C, and *miR-196a2*T>C and for the CCG haplotype of *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G. In meningioma, the odds ratios were increased in the GTCG haplotype of *miR-146a*C>G, *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G. The odds ratios were also increased in the GCG haplotype of *miR-146a*C>G, *miR-196a2*T>C, and *miR-499*A>G and CCG haplotype of *miR-149*T>C, *miR-1962*T>C, and *miR-499*A>G. The odds ratios for schwannoma were increased in the GCTG haplotype of *miR-146a*C>G, *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G and the CCG haplotype of *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G. In conclusion, our results suggested that the miR-149 polymorphism might be involved in the development of gliomas, and the CCG haplotype of *miR-149*T>C, *miR-196a2*T>C, and *miR-499*A>G showed increased odds ratios for all types of brain tumors in Koreans.

CBMT-39. METABOLIC PROFILING OF HUMAN GLIOMAS ASSESSED WITH NMR

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Little is known about the underlying metabolic alterations of gliomas. The objective of this study was to analyze metabolomic profiles of gliomas diagnosed according to revised WHO classification to demonstrate metabolic signatures beyond isocitrate dehydrogenase (IDH) 1/2 mutation. ^{1}H-NMR spectroscopy of tumor extracts was performed to analyze brain tumor metabolism. We detected 46 metabolites including 2-hydroxyglutarate from human brain tumors. Metabolic profiles obtained were analyzed using multivariate analysis and MetaboAnalyst 3.0, a pathway analysis tool. We found that 2-hydroxglutarate, glutamine, and O-phophocholine had top-ranked VIP scores in metabolic pathway analyses of glioma. Major metabolism pathways perturbed in glioma included alanine/aspartate/glutamate metabolism, glycine/serine/threonine metabolism, pyruvate metabolism, taurine/hypotaurine metabolism, and D-Glutamine/D-glutamate metabolism. MetaboAnalyst 3.0 generated three unique clusters of gliomas. Concentration of 4-aminobutyrate was the highest in cluster 1 while concentrations of acetate and O-phosphocholine were the highest in cluster 2. Other metabolites had higher concentrations in cluster 2 compared to those in cluster 1 or cluster 3. Concentrations of isoleucine and phenylalanine were lower in those with high-grade gliomas than in those with low-grade gliomas. Gliomas could be reclassified according to their metabolomics features. Metabolic cluster approach may lead to development of metabolic anti-glioma therapy.

CBMT-40. A SOX2-EXPRESSING PERICYTE PRECURSOR CONSTITUTES A NEW AND EFFICIENT TARGET FOR ANTI-ANGIOGENESIS IN GLIOMAS

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Pericytes are essential for maintaining the stability of CNS blood-vessels and the blood-brain-barrier. They are closely associated with endothelia and can be identified by a set of markers. New pericytes are required during neoangiogenesis but how pericytes are generated in the adult brain is largely unknown. We investigated neoplastic vascularization of the adult brain in a transgenic lineage-tracing model. We observed that the vast majority of mature pericytes originates from a newly identified precursor cell-type. These precursors are characterized as pericyte marker-negative cells of non-hematopoietic origin, which are distant from blood-vessels and highly proliferative. Immunofluorescence analysis of pericyte precursors was corroborated by single-cell transcriptomic data. Pericyte precursors, but not mature pericytes, express the transcription factor SOX2, which has a role in maintaining a stem-like identity. In particular, we observed that traced, SOX2+ cells are confined to a non-vascular localization and do not express pericyte-markers (PDGFRB, Desmin, NG2 or CD146). Whereas mature, traced pericytes were Sox2-negative, expressed the entire set of pericyte-markers and had a perivascular position. Conditional knockout of Sox2 in pericyte precursors resulted in largely reduced numbers of traced cells and blocked the formation of new pericytes. This indicates that new intratumoral pericytes are generated by a previously uncharacterized, Sox2-positive avascular pericyte precursor cell. Lineage ablation experiments in brain tumor models showed that pericyte precursors are necessary for angiogenesis and tumor expansion. All in all, this shows that pericyte precursors are a new and promising target for glioma-therapy.

CBMT-41. GLIOBLASTOMA CLONES DERIVED FROM TUMOR CORE AND EDGE DISPLAY SPATIAL METABOLIC HETEROGENEITY

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The highly heterogeneous nature of glioblastoma (GBM) is a hallmark of limited response to the current therapies and subsequent unfavorable clinical prognosis. Although intratumoral cellular heterogeneity has been recognized in various cancers including GBM, the spatial distributions of tumor-associated cell types and their mechanisms of metabolic pathways that are essential for their heterogeneity remain poorly understood. Here, we utilized the MRI-guided localized biopsy of GBM tumor tissues during surgery, thereby establishing multiple patient-derived GBM clones from both the tumor core and edge tissues in a clonal density, termed core-GBM clones and edge-GBM clones. Using these GBM clones as well as the original tumor tissues, we investigated the underlying molecular mechanisms that drive spatial metabolic heterogeneity in GBM. Comprehensive single cell-metabolome analysis revealed a clear difference between the core and edge clones, as well as between the core and edge tissues. In particular, we noticed metabolic heterogeneity in the nicotinamide adenine dinucleotide (NAD) pathway between the core-GBM clones/tissues and the edge-GBM clones/tissues. Manipulation of the NAD pathway showed distinct phenotypic changes among these clones. These findings suggest that metabolic heterogeneity plays an essential role in therapeutic resistance of glioblastoma.

CBMT-42. LOSS OF PROMOTER METHYLATION IN GLYCOLYTIC GENES IS ASSOCIATED WITH AGGRESSIVENESS IN IDH1-MUTANT LOWER GRADE GLIOMAS

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BACKGROUND: Identification of malignant progression is a major challenge in the field of Neuro-Oncology because of inability of traditional imaging methods to distinguish pseudo-progression from true progression. IDH1-mutant gliomas represent an ideal model system to study the molecular mechanisms associated with malignant progression because they appear initially indolent and non-glycolytic, but eventually a subset progress towards secondary glioblastoma with a Warburg-like phenotype. The mechanisms and molecular features associated with this transformation are poorly understood. METHODS: TS603, NCH1681 and BT142 cell lines harboring IDH1 mutation were cultured in DMEM/F12+ N2 supplement, glutamine, FGF and EGF, then either harvested for metabolomics and methylation analyses or (250,000 cells) were injected into 6-week-old SCID mice brains. MRI was used to monitor tumor size; when tumors reached 100 mm^3, mice were injected in the tail vain with 96 mM 1-^{13}C pyruvate which was hyperpolarized using Oxford Hypersense hyperpolarizer and chemical shift images were acquired immediately. Metabolite quantification was done using the Agilent LC/MS 6545 QTOF mass spectrometer. DNA methylation analysis was performed using Ilumina Infinium MethylationEPIC. RESULTS AND CONCLUSIONS: Cell lines that demonstrate aggressive growth both in vitro and in vivo have lost methylation in the promoters of glycolytic enzymes and have increased mRNA and metabolite levels for the glycolysis pathway compared to the non-transformed model. Metabolic ^{13}C MRI using hyperpolarized^{13}C pyruvate confirmed that these aggressive lines have a Warburg-like phenotype (aerobic glycolysis). Moreover, the glycolytic enzyme expression of the aggressive cell line correlated with the subset of patients that are IDH1 mutated and low-G-CIMP in TCGA database. We hypothesize that specific modulation of epigenetic markers is a mechanism of malignant transformation and that monitoring lactate production may be a biomarker of transformation.

CBMT-43. SINGLE LIVE TUMOR CELL METABOLISM VIA RAMAN IMAGING MICROSCOPY

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BACKGROUND: Raman has been used for decades to determine vibrational models linked with structural changes of small molecules. Due to recent progress in the diode lasers and notch filters, study of biological systems became possible in the form of Raman Imaging Microscopy. Focusing the laser through a microscope allows the collection of Raman spectra for each pixel and subsequent movement of the stage permits the acquisition of spectra in a new position. This approach provides a biochemical mapping of the entire cell while maintaining viability in the culture media. Therefore, the technique is non-destructive, non-invasive and can accept a wide range of samples from bulk to microscopic, from solids to liquids or gasses. However, Raman has not been primarily used for biological samples, therefore spectral assignments for metabolites are currently limited for these samples. METHODS: Tumor cells used for Raman analysis were transferred into 35 mm sterile glass bottom dishes (Ibidi) and cultured for 24 hours. Raman spectra were acquired using a DXR 2xi Raman microscope (Thermo Fisher Scientific) with 24 mW of 780 nm laser through a 60x water immersed confocal objective, at 0.5 s exposure time for a 1 µm pixel size, between 50 and 3200 cm^{-1} spectral region. Spectra were collected and subsequently background corrected using the Raman silent region. Chemical maps were produced by the peak area function using Thermo Fisher Scientific OMNIC software. RESULTS AND CONCLUSIONS: Raman spectroscopy on a single live cells enabled visualization of cytochrome C release from mitochondria after treatment of an inhibitor of glycolyisis drug treatment; quantification of lipid content in a tumor cell upon treatment with AGI-5198 drug, as well as mapping and quantification of DNA damage. These results highlight to potential for Raman imaging to perform single cell metabolomics.

CBMT-44. METABOLIC PLASTICITY AND HETEROGENEITY IN IDH1^{MUT} CELL LINES PRODUCES RESISTANCE TO GLUTAMINASE INHIBITION BY CB839

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BACKGROUND: Mutant IDH1 (IDH1^{mut}) gliomas have characteristic genetic and metabolic profiles and exhibit phenotype that is distinct from their wild-type counterparts. The glutamine/glutamate pathway has been hypothesized as a selective therapeutic target in IDH1^{mut} gliomas. However, little information exists on the contribution of this pathway to the formation of D-2-hydroxyglutarate (D-2HG), a hallmark of IDH^{mut} cells, and the metabolic consequences of inhibiting this pathway. METHODS: We employed an untargeted metabolic profiling approach in order to detect metabolic changes arising from glutaminase (GLS) inhibition treatment. Subsequently, ^{13}C metabolic tracing analysis through a combined Nuclear Magnetic Resonance and Liquid Chromatography-Mass Spectrometry approach, we explored the fate of glutamine and glucose under treatment with CB839 a glutaminase-GLS-inhibitor and their respective contributions to D-2HG formation. RESULTS AND CONCLUSIONS: The effects of CB839 on cellular proliferation differed among the cell lines tested, leading to designations of GLS-inhibition *super-sensitive*, *-sensitive* or *-resistant*. Our data indicates a decrease in the production of downstream metabolites of glutamate, including those involved in the TCA cycle, when treating the sensitive cells with CB839 (glutaminase -GLS- inhibitor). Notably, CB839-*sensitive* IDH1^{mut} cells respond to GLS inhibition by upregulating glycolysis and lactate production. In contrast, CB839-*resistant* IDH1^{mut} cell lines do not rely only on glutamine for the sustenance of TCA cycle. In these cells, glucose contribution to TCA is enough to compensate the downregulation of glutamine-derived TCA metabolites. This investigation reveals that the glutamine/glutamate pathway contributes differentially to D-2HG in a cell-line dependent fashion on a panel of IDH^{mut} cell lines. Further, these results demonstrate that there is a heterogeneous landscape of IDH1^{mut} metabolic phenotypes. This underscores the importance of detailed metabolic profiling of IDH1^{mut} patients prior to the decision to target glutamine/glutamate pathway clinically.

CBMT-45. A NOVEL C-CIRCLE ASSAY FOR DETECTING ALTERNATIVE LENGTHENING OF TELOMERES (ALT) MECHANISMS

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Low-grade gliomas, in particular astrocytoma, have been previously reported to harbor Alternative lengthening of telomere (ALT) to maintain their telomeres to aid sustained replication. Targeting genetic pathways specific to ALT mechanism is crucial in developing novel drugs to minimise recurrence and improve prognosis in low grade glioma patients. Herein, we used a novel C-circle assay to identify ALT positivity in a local, retrospective cohort of astrocytoma and oligodendroglioma, where matched primary and recurrent tumors were available. We identified a retrospective cohort of 25 patients with grade II or III astrocytomas (n=17) or oligodendrogliomas (n=8) where matched tissue from recurrences were available. DNA was extracted and a novel C-circle assay was used to determine the ALT status of the primary and recurrent tumors. Approximately 77% (13 out of 17) astrocytomas were positive for ALT. Only 25% (2 out of 8) oligodendrogliomas were ALT positive. LOH 1p/19q was confirmed in these cases. Patients who were ALT positive showed longer overall survival. We found a significant association with ALT and IDH1 mutation status and mutations in TP53. In conclusion, the C-circle assay provides a robust and quantitative measure of ALT and could be incorporated into routine clinical use.

CBMT-46. MICROENVIRONMENT-DERIVED MITOCHONDRIA PRIME GLIOMA CHEMORESISTANCE BY AUGMENTING NAD+ METABOLISM AND PARP-DEPENDENT DNA REPAIR

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BACKGROUND: Mitochondria is the major organelle for cellular metabolism. Increasing evidence shows that mitochondria are released into the extracellular microenvironment and taken up by adjacent cells, reshaping the metabolism and other biological processes in the recipient cells. While knowledge of intercellular mitochondria transfer under normal physiology is expanding, the roles of mitochondria transfer in tumor microenvironment and disease outcome remains elusive. METHODS: In this study, we established *in vitro* and *in vivo* models to aid in the understanding of mitochondrial transfer between astrocytes and glioma cells. We investigated the mitochondria population in extracellular vesicles (EV), and their influence on the recipient glioma cells, with focuses on NAD^+ metabolism and PARP DNA repair pathway. The role of intercellular mitochondria was further validated in glioma xenograft model with chemotherapy. RESULTS: We demonstrated that mitochondria are released from normal astrocytes through EV and adopted by neighboring glioma cells. Moreover, mitochondrial transfer improved metabolism in recipient glioma cells, supporting the detoxification of chemotherapeutic agents and evoking treatment resistance. Mechanistically, mitochondria-derived NAD^+ metabolic enzymes expanded the pool of NAD^+ in recipient cancer cells, which fueled PARP DNA repair pathway with key cofactors. Targeting astrocyte mitochondria-releasing pathways not only increased the chemotherapy-derived cytotoxicity, reduced xenograft progression, but also improved disease outcome by prolonging overall survival. CONCLUSION: Our findings revealed that microenvironment-derived mitochondria transfer is a potential chemoresistance mechanism in cancer cells. Targeting intercellular mitochondria transfer may be important in reducing the capacity for cancer cells to recover from cancer treatment, thereby enhancing treatment efficacy.

CBMT-47. THE ROLE OF PHOSPHOFRUCTOKINASE-1 IN GLIOBLASTOMA MAINTENANCE AND MOTILITY

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BACKGROUND: Glioblastoma (GBM) remains one of the most lethal tumors and is associated with a median survival of only approx. 15 months despite aggressive radio-chemo-therapy. One distinct challenge in GBM management includes diffuse location of the tumor within the brain and migration of cancerous cells into the healthy surrounding tissue preventing complete surgical removal and promoting recurrence. A subpopulation within the tumor mass, the so-called stem cells (or glioma initiating cells, GICs) are further complicating treatment strategies as this pool of cells is more invasive and is known to possess resistance to traditional therapy. It is commonly known that cancer cells utilize glucose as their main energy source, a phenomenon known as the Warburg effect. Phosphofructokinase-1 (PFK-1) is one of two rate-limiting enzymes in glycolysis and presents a potential critical check point for these glucose "addictive" cells. Additional to its glycolytic role we here propose a regulatory role for PFK-1 in motility in GICs. METHODS: To elucidate the role of PFK-1 in GBM, we abrogated

its function using shRNA-mediated knockdown or chemical inhibition. Next, we evaluated the cellular responses utilizing a broad range of in vitro assays including cell viability, apoptosis, cytoskeleton assembly, migration and invasion assay. RESULTS: Depletion of PFK-1 in GICs led to decreased viability and increased rates of apoptosis. Furthermore, impaired function of PFK-1 affected cytoskeleton assembly and decreased migration and invasion capacity, a phenotype we were able to mimic with a chemical PFK-1 inhibitor. PFK-1-regulated motility was found to be through translational regulation of KIF11. CONCLUSION: PFK-1 regulates GBM stem cell motility and invasion through the regulation of KIF11, a motor protein belonging to the kinesin-like protein family. The novel role of PFK-1 identified in our studies, together with successful therapeutic targeting in vitro and in vivo, expands our understanding of GBM maintenance and cell motility.

CELL SIGNALING AND SIGNALING PATHWAYS

CSIG-01. GLYCOPHOSPHATIDYLINOSITOL TRANSAMIDASE (GPIT) SUBUNIT GPAA1 IS OVEREXPRESSED IN GLIOBLASTOMA MULTIFORME (GBM) CELL LINES AND CONTRIBUTES TO TUMOR CELL MIGRATION

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The glycophosphatidylinositol (GPI) anchor is a highly conserved lipid and glycan modification added to the C-terminus of substrate proteins in the endoplasmic reticulum via the activity of the multi-subunit GPI transamidase, or GPIT. Several subunits of the GPIT, namely *GPAA1*, have previously been characterized as oncogenes across a variety of tumor types. Using a *C. septicum* alpha toxin enrichment strategy pioneered by our lab, we have previously documented significantly elevated plasma levels of GPI anchored proteins occurring in glioblastoma (GBM) patients relative to non-malignant controls. To better understand the biological significance of GPIT expression and subsequent activity in GBM, we have combined the use of qRT-PCR, immunoblot analysis, chemotaxis and clonogenic assays, with targeted alpha toxin glycoproteomics. Using these methods, we have documented significant increases in GPIT subunit expression in human GBM cell lines relative to normal human astrocyte controls. The observed increases in GPIT expression levels corresponded with increases in GPI anchored protein content. In addition, we found GPAA1-targeted knockdown in U87-MG is sufficient to significantly decrease both tumor cell migration and clonogenic cell growth relative to non-targeted control U87-MG. Collectively, these results suggest that GPIT and GPI anchors are involved in the tumorigenicity of GBM and may represent novel therapeutic alternatives in this disease.

CSIG-02. VAL-083 INHIBITS PROLIFERATION OF A PANEL OF EIGHT GLIOBLASTOMA STEM CELL LINES: DOWNREGULATION OF BDR4 AS A NOVEL ANTI-NEOPLASTIC MECHANISM

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VAL-083 (Dianhydrogalactitol) is a bi-functional DNA targeting agent that is currently being evaluated in a phase II trial in recurrent glioblastoma (GBM) patients. The goal of the present study was to further elucidate the anti-neoplastic effects and signaling pathways through which VAL-083 functions. We examined the efficacy of VAL-083 against a panel of eight GBM stem cells (GSCs) isolated from newly diagnosed GBM patients. The panel of GSCs were molecularly phenotyped based on the expression of several proteins including EGFR, EGFRvIII, and MGMT as well as several stem cell markers including SOX2, NESTIN, MST1, CD133, TFRC, and OLIG2. The effect of VAL-083 on GSC growth was measured using WST-1 reagent and the effect on GSC's ability to form neurospheres was assessed by microscopy. Our results show that VAL-083 inhibits neurosphere formation in all eight GSCs. Further, VAL-083 inhibits the growth of GSCs with an IC_{50} of 200–2000 nM. To identify the molecular pathways affected by VAL-083, control and VAL-083-treated GSCs were subjected to proteomic analysis using reverse phase protein array (RPPA) technology. The RPPA examined the expression of a total of 297 proteins and phosphoproteins. It was found that VAL-083 affects the expression of several proteins and phosphoproteins central to GBM growth. A key protein significantly downregulated by VAL-083 was bromodomain protein 4 (BRD4). This is a salient important finding because BRD4 has been implicated in several cancers including GBM, and agents that target BRD4 are undergoing development as anti-neoplastic agents. In summary, we report that VAL-083 is effective in halting the growth of a panel of GSC isolated from newly diagnosed GBM patients and the underlying mechanism involves downregulation of BRD4.

CSIG-03. CYCLIC DEPSIPEPTIDE LIGANDS TO TARGET CO-TRANSLATIONAL TRANSLOCATION AND ER PROTEOSTASIS IN GLIOBLASTOMA CELLS

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Coibamide A, is a rare cytotoxic N-methyl-stabilized cyclopeptide originally isolated from a cyanobacterium growing within the marine reserve of Coiba National Park, Panama. We have recently determined that this natural product targets the co-translational translocation machinery and potently inhibits expression of secreted, resident endoplasmic reticulum (ER) and membrane-bound proteins. This mechanism of coibamide A action leads to a pattern of cellular consequences including inhibition of glucose regulated protein 78 (GRP78) expression and a specific pattern of cell stress and death signaling in cultured glioblastoma cells. Exposure to nanomolar concentrations of coibamide A, or the related cyclic depsipeptide natural product apratoxin A, promotes proteasomal degradation of GRP78 and a compensatory upregulation of cytosolic heat shock proteins 40 and 70 that precedes cell death. Co-translational translocation is mediated by the Sec61 translocation channel, which comprises a conserved hetero-oligomeric protein composed of a main pore-forming Sec61alpha subunit plus beta and gamma subunits. Sec61 also signals directly with the protein folding machinery of the ER lumen to maintain proteostasis. The Sec61 translocon channel is not a direct target of any currently approved or experimental drug, however, several natural product structures are now known to target the Sec61 channel and inhibit the co-translational translocation process. Pharmacological inhibitors of Sec61 may be valuable tools to probe GBM biology as *SEC61gamma* has previously been identified as a proto-oncogene, and Sec61gamma overexpression reported in high versus low grade glioma or normal astrocytes. Natural products have historically been important sources of new chemical structures, particularly for cancer and infectious disease, rather than providing the final drug entity. Although preliminary, the discovery of natural product ligands to target the ER translocation machinery reveals a distinct mechanism to perturb proteostasis in aggressive CNS cancers characterized by high expression of GRP78 and therapeutic resistance.

CSIG-04. A REQUIREMENT FOR RIOK2 CATALYTIC ACTIVITY IN RTK-PI3K DEPENDENT GLIOBLASTOMA

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Glioblastoma (GBM) is a particularly lethal brain neoplasm that accounts for half of malignant and fifteen percent of all primary brain tumors diagnosed in adults. These statistics elevate GBM to a priority in neuro-oncology research, but the tumors remain incurable with an exceptionally poor median survival of fifteen months after diagnosis. In order to promote targeted therapy development, there are ongoing efforts to understand GBM at a molecular level, which involve characterization of these tumors via their transcriptional and mutational profile. Recent studies indicate aberrations in receptor tyrosine kinase (RTKs), including EGFR and PDGFRA, and the Pi-3 kinase (PI3K) signaling pathways are major drivers of tumorigenesis in GBM. Using a *Drosophila melanogaster*GBM model to locate downstream targets of these pathways, our laboratory has identified right open reading frame 2 (RIOK2) serine-threonine kinase as a downstream effector of aberrant RTK-PI3K signaling in glial tumorigenesis. Subsequent follow-up studies indicate that RIOK2, which is overexpressed in RTK mutant human GBMs, drives survival and proliferation of RTK-PI3K-dependent human GBM cells. In order to understand how RIOK2 functions to promote tumorigenesis through RTK-PI3K signaling, we used a chemical genetics approach in both *Drosophila*and cultured human GBM cells to test the effects of catalytic inhibition of RIOK2 in tumorous glia as well as normal glia to find that RIOK2 catalytic activity is essential for the growth of tumor cells, but dispensable for the growth of normal glia. However, the substrates of RIOK2 are unknown. Therefore, through a combination of proteomic and genetic approaches, we are currently seeking to identify potential targets of RIOK2 catalytic activity. Together, our data indicate a role for RIOK2 in promoting GBM tumorigenesis and our ongoing studies reveal an important tumor-specific target signaling pathway for consideration in experimental therapeutics.

CSIG-05. PI3K INHIBITORS PX-866 AND BEZ235 DIFFERENTIALLY MODULATE AUTOPHAGY IN GBM

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Glioblastoma multiforme (GBM) is the most common and deadly neurological malignancy with few treatment options. Maximal safe surgical resection of the primary tumor followed by radiotherapy (RT) with concomitant

Temozolomide (TMZ) remains the primary standard of care, but in all cases, the tumor eventually recurs. The recurrent tumor typically harbors a distinct molecular landscape compared to the primary tumor and displays resistance to RT and TMZ. With no effective treatment options for recurrent tumors that prolong lifespan, there is a great need to understand the molecular alterations that have occurred in order to address resistance and provide treatment options. One pathway that has been previously reported to be up-regulated in recurrent tumors is the PI3K/AKT/mTOR axis, which has prompted the use of various PI3K inhibitors in the clinic. Unfortunately, these have shown to be largely ineffective either as stand-alone agents or in combination with other precision-targeted drugs. Here, we have explored the action of 2 PI3K inhibitors; PX-866 (pan PI3K inhibitor) and BEZ235 (dual PI3K and mTOR inhibitor) and their ability to modulate the pro-survival autophagy pathway in T98G cells and the recurrent patient-derived xenograft (PDX) cell model GBM10. Both PX-866 and BEZ235 showed strong inhibition of the PI3K/AKT/mTOR axis. However, in combination with the autophagic blocker Bafilomycin A1, PX-866 inhibited autophagic flux and BEZ235 induced autophagic flux. Interestingly, both compounds showed an increased activation of ERK signaling, potentially leading to an altered dependence on MAPK signaling when the PI3K/AKT/mTOR pathway is inhibited. The differential effects on autophagy and the induction of ERK signaling using PX-866 and BEZ235 can be used to further provide rational drug combinations for the treatment of recurrent GBM, while still effectively targeting the overactive PI3K pathway, which GBM heavily relies on for survival and invasion.

CSIG-06. THE MOLECULAR SUBTYPE OF PRIMARY GLIOBLASTOMA CELLS CORRELATES WITH RESPONSE TO THERAPEUTIC AGENTS THAT INDUCE APOPTOSIS OR SENESCENCE

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INTRODUCTION: Glioblastoma (GBM) is the most common adult primary brain tumour. Despite maximal therapy, median survival is 14 months. Resistance to therapy in GBM is due to extensive molecular heterogeneity. Gene expression profiling demonstrates four major subtypes: proneural, neural, classical, and mesenchymal. Recently it was shown that the mesenchymal subtype of GBM cells are resistant to ionizing radiation induced apoptosis. However, the response of other subtypes to therapy with respect to apoptosis or senescence (irreversible growth arrest) remains unknown. Further investigation into the susceptibility of the molecular subtypes and mechanisms responsible for resistance may yield insight into novel therapeutic targets. METHODS: Primary Glioblastoma (PriGO) cells were harvested from 3 human patients with GBM and cultured in serum free media. Microarray analysis was used to determine the predominant molecular subtype of each cell line. Cells were then treated with radiation, chemotherapy, or serum (an agent known to induce senescence in PriGO cells). Apoptosis was measured by cell counts, caspase-3 activation, and Annexin-V positivity. Senescence was determined by SA-β-Gal assay, markers of cell cycle arrest (p21) and heterochromatin formation (PML bodies). RESULTS: PriGO8A and PriGO9A cells were predominantly classical whereas PriGO17A cells were predominantly mesenchymal. Classical PriGO8A and PriGO9A cells underwent apoptosis in response to radiation and the chemotherapeutic agent Triapine but underwent senescence in response to serum. Mesenchymal PriGO17A cells failed to undergo apoptosis or senescence in response to any agent. Inhibition of a key hyperactive pathway in mesenchymal cells, the Ras pathway, led to an increase in senescence induction. CONCLUSIONS: The molecular subtype of GBM correlates with response to therapy. The classical subtype is sensitive to agents that induce apoptosis and senescence whereas the mesenchymal subtype is resistant. Resistance to therapy may be mediated by the Ras pathway and its inhibition may render such cells susceptible to senescence inducing agents.

CSIG-07. COMPARATIVE RNA-SEQ ANALYSIS OF GLIOMAS OF DIFFERENT MALIGNANCY IDENTIFIES FYN AS A NOVEL REGULATOR OF GBM AGGRESSIVENESS

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Glioblastomas (GBM) are the most frequent and aggressive primary tumors of the brain. Fyn, a Src family kinase member, is overexpressed in human GBM. Its function, however, remains poorly understood. We analyzed the differential gene expression (DE) of highly malignant tumor (NPA: N-Ras/shp53/shATRx) compared to a less malignant one (NPAI: N-Ras/shp53/shATRx/IDH1R132H). Bioinformatics and network analysis identified Fyn as a highly connected node. This suggests that Fyn could be a regulator of GBM growth and progression. We therefore investigated the role of Fyn on glioma function in vitro and in vivo. Fyn expression levels in both human and mouse GBM cells correlated with tumor aggressiveness. Fyn knockdown in NP and NPA glioma cells decreased cell proliferation and migration. To test the activity of Fyn on tumor growth in vivo, we developed a Fyn-deficient glioma genetic model using the Sleeping Beauty transposase system. We induced Fyn knockdown in glioma tumors with different genetic drivers: NPF: N-Ras/shp53+**shFyn**, NPAF: N-Ras/shp53/shATRx+**shFYN** and NPDF: N-Ras/shp53/PDGFβ+**shFYN**. Fyn knockdown increased survival in all three genetically different tumors. The absence of Fyn reduced malignant neuropathological features such as pseudopalisades, ischemic necrosis, and microvascular proliferation. At the molecular level, RNA-Seq and DE analysis comparing NPF tumors with their controls, NP tumors (N-Ras/shp53) identified 573 differentially expressed genes. Bioinformatics analysis indicated altered activity in the following Gene Ontologies: "regulation of cell adhesion", "inflammatory response", and "positive regulation of cell motility". In addition, signaling pathways significantly altered in their activity included "cell adhesion molecules (CAMs)", "focal adhesion" and "ECM receptor interaction". Our results indicate that Fyn would exert its effects by regulating cell motility and interactions with the ECM. We suggest that inhibiting Fyn activity, a novel regulator of glioma malignancy, could become a relevant treatment for GBM.

CSIG-08. DYNAMICS OF GLIOMA GROWTH: SELF-ORGANIZATION GUIDES THE PATTERNING OF THE EXTRACELLULAR MATRIX AND REGULATES TUMOR PROGRESSION

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GBM remains the deadliest primary malignant brain tumor. Given the importance of invading cells, less attention has been paid to the tumor mass, even if such a mass eventually kills the patient. We previously demonstrated that human and mouse transplantable or GEMM gliomas display regular anatomical multicellular structures containing elongated cells which we named 'oncostreams'. Oncostreams are 10–20 cells wide, 2–400 um long, and are distributed throughout the tumors. Furthermore, we uncovered a negative correlation between oncostream density and animal survival suggesting that oncostreams play a role in tumor malignancy. Further data indicate that oncostreams aid local invasion of normal brain. Co-implantation experiments demonstrated that oncostreams facilitate the intratumoral spread of slow migrating cells. To determine a possible molecular distinctiveness of oncostreams we used laser capture microdissection followed by RNA-Seq, bioinformatics and network analysis. Evaluation of the transcriptome demonstrated differential expression (DE) of genes between oncostreams and adjacent tumor. Functional enrichment of DE genes showed that "collagen catabolic processes", "positive regulation of cell migration", and "extracellular matrix organization" were the most over-represented gene ontologies. Network analysis indicated that Col1a1, ACTA2, MMP9, MMP10 and ADAMTS2, genes important for cell migration and ECM interactions, are part of these networks. IHC and PCR were used to validate RNA-Seq expression changes. To understand the cellular dynamics in our system we used time lapse imagining and evaluated the results using high level statistical analyses. The following tests, i.e., velocity distribution, pair-wise correlation of local position, velocity correlation, etc. were used to characterize the dynamics of cellular intratumoral motility. These data demonstrate the existence of collective motion within glioma tumors. Taken together, our results show that oncostreams, move by collective motion, are anatomically and molecularly distinct, reveal the existence of glioma self-organization, and regulate glioma growth and invasion. Targeting oncostreams is a candidate therapeutic strategy.

CSIG-09. THE ROLE OF HYDROGEN SULPHIDE IN GLIOMAS

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Glioblastoma is the most common malignant brain tumour in adults, with high relapse rates and a dismal prognosis. Despite advances in our understanding of the pathobiology of the condition, a curative treatment has yet to be found. Hydrogen sulphide (H_2S) is a multifunctional novel gasotransmitter, which has recently garnered interest in the tumour biology field. However, little is known about this molecule in glioblastoma. A systematic review of the literature was thus undertaken to determine whether H_2S and its synthesizing enzymes play a significant role in the glioma tumour biology. A total of 108 published articles were identified in our systematic search of electronic databases (PUBMED, WEB OF SCIENCE). After screening for eligibility, a total of seven studies investigating the role of sulphur, H_2S and H_2S synthesizing enzymes underwent comprehensive review, quality assessment, and data extraction. H_2S synthesising enzymes CBS, CSE and MPST were

increased in glioma cells compared to normal non-tumour cells in all the studies. The MPST enzyme appears to be particularly abundant in glioma cells. The CBS enzyme was shown to have a beneficial role in glioma, and when deficient led to increased angiogenesis and tumour growth. H_2S produced from cysteine is stored as bound sulphane sulphur. Levels of sulphane sulphur was shown to be raised in high grade gliomas compared to normal human brain regions and were thought to be involved in tumour proliferation. The underlying mechanisms of H_2S -induced effects in glioma cells were investigated in only three studies. Although there is a clear evidence of the presence of H_2S or its synthesising enzymes, the role of H_2S in glioma has not been fully delineated. A thorough understanding of the molecular mechanisms underlying its action in glioma and in vivo studies investigating its affect on tumour growth and survival are warranted.

CSIG-10. INVESTIGATING THE S6K1 AND S6K2 IN PTEN-DEFICIENT GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM), the most lethal type of malignant brain cancer in adults, sustains frequent mutations and/or deletions in the tumor suppressor gene PTEN (phosphatase and tensin homolog), resulting in the activation of downstream mTOR-S6K signaling. METHODS: We proposed experiments to test the hypothesis that PTEN deficiency induces signaling through both S6K1 and S6K2. We used for genome editing by knock down of S6K paralogs in combination with LY2584702, an S6K1 inhibitor. RESULTS: We found that LY2584702 is highly specific for S6K1 inhibition in a kinome-wide analysis. Consistent with this result, gene-targeted S6K1-deficient cells exhibited little additive effect when treated LY2584702. S6K2 knockout induced a substantial reduction in the phosphorylation of the S6K substrate, ribosomal protein S6 (rpS6). Addition of LY2584702 reduced rpS6 phosphorylation further, indicating that S6K2 cooperates with S6K1 to mediate pathway signaling downstream of mTORC1 in PTEN-deficient glioma cells. Similar results employing genetic S6K1/S6K2 targeting using combination siRNA and sgRNA approaches are consistent with the results of LY2584702 experiments. We survey additional S6K substrates to define the relative contributions of S6K paralogs in signaling induced by PTEN-deficiency. CONCLUSIONS: In short, our data support overlapping features of S6K1 and S6K2 regulation by PTEN, suggesting the importance of both kinases as clinical targets in GBM.

CSIG-11. UNDERSTANDING THE MECHANISM OF RIOK2 FUNCTION IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is the most aggressive and prevalent form of primary brain cancer and is incurable. Amplification, mutation, and/or overexpression of the EGFR receptor tyrosine kinase and activating mutations in components of the PI3K pathway are common in GBM tumors, although the pathways that act downstream of EGFR and PI3K to drive tumorigenesis remain poorly understood. To better understand the underlying biology of tumorigenesis, we use a *Drosophila melanogaster* GBM model in which malignant neoplastic tumors arise from glial progenitor cells overexpressing activated oncogenic versions of EGFR and PI3K and identified Right-Open-Reading-Frame-2 (RIOK2), an atypical serine-threonine kinase, as a possible driver of EGFR-PI3K-dependent GBM. To elucidate downstream targets of RIOK2, we conducted preliminary immunoprecipitation experiments of RIOK2 from patient-derived GBM cell cultures coupled with proteomics and identified several novel RNA-binding proteins (RBPs) as binding partners and potential substrates of RIOK2. Subsequent experiments using our *Drosophila* GBM model show that RBP knock-down drastically reduced aberrant glial cell proliferation and invasion, similar to RIOK2 knock-down. Furthermore, co-immunoprecipitation experiments using patient derived primary GBM cells reveal that RIOK2 and novel RBPs are co-IPed and may operate in a complex. Moreover, RNA-binding protein immunoprecipitations (RIP) suggest that RIOK2 is associated with a number of oncogenic mRNAs. Based on our preliminary results, we hypothesize that RIOK2 drives tumorigenesis by modulating the activity of RBPs, and that this promotes the translation of RBP target mRNAs to drive tumor cell proliferation and survival.

CSIG-12. ANNEXIN A7 ISOFORMS DIFFERENTIALLY REGULATE TUMORIGENIC EGFR SIGNALING IN GLIOBLASTOMA MULTIFORME

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Dysregulation of receptor tyrosine kinase genes is a frequent genetic abnormality in GBM. A common genetic alteration is amplification and/or overexpression of the *epidermal growth factor receptor* (*EGFR*) gene. Annexin A7 (ANXA7) is a hydrophilic protein that binds membranes in a calcium dependent manner; it is important for membrane scaffolding and vesicle trafficking. *ANXA7* is alternatively spliced and expressed as two isoforms – unspliced *ANXA7* Isoform 1 (I1), containing cassette exon 6 and spliced Isoform 2 (I2). Our lab has determined that I1 is tumor suppressive in GBM and that loss of I1 promotes tumorigenicity by perpetuating EGFR signaling. However, if restored, I1 but not I2, reduces EGFR levels, activation and downstream pathways and diminishes tumorigenicity. In patients, I1 levels directly correlate with survival and prognosis. We propose that I1 inhibits EGFR signalling by binding to and promoting degradation of EGFR in a calcium dependent manner. In GBM, I1 is absent, preventing endosomal degradation of EGFR and leading to perpetual signalling. Our approach is novel; rather than address how/whether EGFR is activated, we focus on why EGFR fails to be downregulated afterwards. Herein, we present a mechanism that explains, in part, how I1 mediates endosomal degradation of EGFR. Further studies are underway to identify exactly where and how I1 modifies endosomal trafficking and degradation of EGFR. We are also exploring the role of I1 in regulating the degradation of other receptor tyrosine kinases (RTKs), such as MET and PDGFRA. Collectively, understanding how I1 functions will provide the foundation for future, selective therapies that restore I1 expression and/or function to reduce GBM tumorigenicity.

CSIG-13. COMPUTATIONAL MODELING OF GLIOBLASTOMA STEM CELL SIGNALING NETWORKS

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Despite a tremendous increase in knowledge about glioblastoma in recent years, it has proven difficult to devise new effective therapies. It is likely that a major reason for the failure of new therapies is due to the molecular heterogeneity of GBM between tumors. We have found from RNAi screens that there is significant diversity in essential genes between the tumor stem cells of different patients with only about 50% of all inhibitory kinases being in common between any 2 stem cell lines. Thus, it is likely that a personalized therapeutic approach will be needed for effective treatment of brain tumors. We are building causal computer models of signaling pathways and networks in these tumor stem cells in order to predict the drug responsiveness of individual GBM stem cell lines. To do this, we are using a framework that assembles and tests element rule-based models in an automated manner, assuming a discrete modeling approach, capable of capturing causal relationships between model elements. The use of causal relationships (positive and negative regulation), in addition to mechanistic relationships (e.g., phosphorylation, binding), allows for capturing not only direct but also indirect interactions between elements. By including these indirect interactions on pathways, when there is no information available about exact mechanisms, we are able to account for known element relationships, and capture larger network motifs (e.g., intertwined feedback and feedforward loops), which are often critical in network response to interventions. Our current model with 141 elements and 298 edges can successfully model responses to CDK6 and GSK3-beta inhibition after initialization with RNA-Seq data. With our automated framework, we are now extending the model using text mining with human supervision, and we will test the extended model against chemical inhibitor and RNAi data from multiple GBM stem cell lines.

CSIG-14. ALPHA-CARDIAC ACTIN 1 IS EXPRESSED IN MEDULLOBLASTOMA AND MAY PLAY A ROLE IN CELL SURVIVAL DURING MITOTIC INHIBITION

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We have previously demonstrated that Myc-overexpressing medulloblastoma cells are sensitive to apoptosis induction in response to mitotic inhibition with an Aurora kinase B inhibitor. Profiling of differential mRNA expression by microarray and RT-PCR revealed that alpha cardiac actin 1 (*ACTC1)* mRNA expression is upregulated in cells resistant to apoptosis triggered by Aurora kinase B inhibition and this upregulation is absent in Myc-overexpressing cells. We confirmed expression of ACTC1 protein by Western blot in SHH subgroup (DAOY, UW228, UW426) and Group 3 subgroup cell lines (D458, D425) and observed that expression is lower in Group 3 cells. These findings were further validated by analysis of *ACTC1* mRNA expression among all four medulloblastoma subgroups by microarray in a set of 763 medulloblastomas which revealed increased expression of *ACTC1* in SHH and WNT subgroups compared to Group 3 and Group 4. Inhibition of Aurora kinase B in SHH cells that overexpress Myc results in a reduction in ACTC1 protein level after 96 hours and this is not observed in the isotype control cells with basal Myc expression. Reduction in ACTC1

protein levels in SHH cells overexpressing Myc is associated with Caspase 3 cleavage. ACTC1 may play a role in blocking apoptosis triggered by mitotic inhibition in medulloblastoma. Further experiments to test this hypothesis are planned. These finding could potentially impact on chemotherapy choice in the treatment of SHH and WNT tumours, which demonstrate increased ACTC1 expression.

CSIG-15. SLFN5: A REPRESSOR OF INTERFERON-INDUCED SIGNALING THAT STIMULATES GLIOBLASTOMA CELL PROLIFERATION. SURVIVAL RESPONSES IN DIFFERENT XENOGRAFT GLIOBLASTOMA MOUSE MODELS

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The Schlafen (SLFN) family of proteins are involved with essential cellular functions in normal as well as tumor biology. In our previously published work (Oncogene 2017;36:6006–6019) we showed that high-level expression of SLFN5, an IFN-regulated member of the SLFN protein family, was correlated with shorter overall survival in glioblastoma (GBM) patients and that SLFN5 is a contributor to the aggressive biology of GBM in stimulating the proliferation, motility and invasiveness of GBM cells, as indicated by the results of in vitro assays. In that study we had also found that type-I IFN treatment triggers SLFN5 interaction with STAT1, and this suppresses STAT1-mediated gene transcription. Thus, SLFN5 is a repressor of IFN-gene transcription, suggesting the existence of a feedback loop that may contribute to the suppression of antitumor immune response. Here, we have examined the engraftment and xenograft growth effects of U87 SLFN5 KO cells. Wild-type (wt) and KO cells were intracranially injected in athymic nu/nu mice as well as NSG-SGM3 mice: the former are T-cell deficient whereas the latter are deficient in T-, B-, and NK cells. Athymic and NSG-SGM3 mice injected with KO cells lived significantly longer than mice injected with wt cells: all two-way comparisons yielded log-rank p-values of 0.011 or less. Interestingly, the survival of NSG-SGM3 mice injected with KO cells was significantly reduced relative to athymic nu/nu mice injected with the same cells: $p = 0.027$. This differential response raises the possibility of a potential immunosuppressive effect of SLFN5 involving NK and B-cells. Immunohistochemical analysis of intracranial xenograft tumors for KO effects on proliferation, apoptosis, and immune cell infiltrates is ongoing, and will be presented at the meeting.

CSIG-16. INTRONIC miR-744 INHIBITS GLIOBLASTOMA INVASION THROUGH INHIBITION OF MAPK-, SMAD- AND BETA-CATENIN SIGNALING

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INTRODUCTION: There is accumulating evidence that intronic microRNAs (miRNAs) are capable of either supporting or restraining functional pathways of their host genes thereby creating intricate regulative networks. We hypothesized that such type of interaction might gain special importance in human glioblastoma (GBM): The second intron of Mitogen-Activated Protein Kinase Kinase 4 (MAP2K4), an important hub in the pro-invasive MAPK pathway, harbors miR-744. We hypothesized that miR-744 regulates GBM migration by interacting with its host´s pathways. METHODS: MiR-744 was stably overexpressed in U87 cells. U87 invasion was studied using migration and Boyden chamber assays. TGFB1, MAP2K4 and DVL2 levels were measured by quantitative Real-Time-PCR (qRT-PCR) and SDS-PAGE. Interactions of miR-744 and 3`UTRs were analysed by luciferase reporter assays, SMAD2/3, p38 and beta-Catenin activity by SDS-PAGE, and TOP/FOPflash reporter gene assays. Stereotactically obtained tumor specimens were analysed by qRT-PCR. Experiments were performed in triplicate at least 3 times. Results are presented as mean±SEM. P-Values were calculated using Student's t-test. RESULTS: MiR-744 overexpression inhibited U87 invasion in migration- and Boyden chamber assays (-46%±5,8%, n=4; p=0.026), qRT-PCR and SDS-PAGE revealed reduced levels of TGFB1, DVL2 and MAP2K4 (mRNA:-38%±5.6%, -33.6%±4.9% and -66.2%±7.9%; protein:-35.6%±8.3%, -36.8%±5.3% and -56.2%±9.6%; n=5, p0.05). TGFB1 knockdown repressed MAP2K4 mRNA- and protein levels (mRNA:-66.2% ±7.9%, protein:-56.2% ±9.6%, n=5, p<0.05). MiR-744 levels were dramatically decreased in glioblastoma samples (-90.3%±14.7%, n=21, p<0.05). DVL2 and TGFB1 were significantly induced (DVL2:3.35-fold±0.14; n=17; p<0.001; TGFB1:2.19-fold±0.09, n=29, p=0.015). CONCLUSION: These results provide evidence that miR-744 acts as an intrinsic brake on its host: It impedes MAP2K4 functional pathways through simultaneously targeting SMAD, beta-Catenin and MAPK signaling networks, thereby strongly mitigating pro-migratory effects of MAP2K4. Reexpression of miR-744 could be a promising approach to attenuate GBM invasion.

CSIG-17. CHARACTERIZATION OF AN ALTERNATIVELY SPLICED NTRK2 VARIANT IN GLIOMA: EMPLOYING NOVEL REAGENTS TO UNCOVER NOVEL FUNCTIONS

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Most known for their essential roles in the development and maintenance of the nervous system, the neurotrophins consist of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), NT-3, NT-4 and their respective tropomyosin receptor kinases (TRKs) TrkA, TrkB, and TrkC along with the low affinity nerve growth factor receptor, p75. In addition to known roles in neuronal survival, proliferation, differentiation, and apoptosis, TRKs exert diverse effects on cellular outcomes through their interactions with downstream signaling cascades. Prior to TRKs' established roles in neurobiology, oncogenic TRK was discovered as tropomysoin 3 (TPM3)-TrkA fusion and as a result, oncological studies of the neurotrophin family have identified additional TRK fusions. Putative oncogenic TRK fusions have been observed in various cancer types, but their clinical significance remains unclear and these fusions tend to occur at very low frequencies below 1–2%. The low incidence of these fusions combined with significant overexpression of various TRKs in a multitude of cancers raises the possibility that another aspect of TRK biology, in addition to kinase-domain fusions, may be relevant. Basic scientific and clinical investigation surrounding TRKs' role in cancer has often been hindered due to the nonspecific nature of antibodies and kinase inhibitors, combined with a lack of precise exon-specific expression data from patient populations. Tropomyosin receptor B (TrkB), encoded by the NTRK2 gene, exhibits complex alternative splicing patterns. Here we show a novel role for a TrkB splice variant in gliomas via NTRK2 transcript analyses and immunostaining using a novel antibody engineered specifically to this variant. This NTRK2 splice variant enhances PDGF-driven gliomas *in vivo* and augments PDGF-induced signaling *in vitro*. Through the lens of NTRK2, these results highlight the importance of expanding upon whole gene-level and kinase-fusion analyses to explore TRK splicing in basic and translational research.

CSIG-18. MODELING TEMOZOLOMIDE RESISTANCE WITH GLIOBLASTOMA PATIENT DERIVED XENOGRAFTS

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We have conducted experiments with intracranial patient-derived xenograft (PDX) models of glioblastoma (GBM) in which PDX recurrence, following the administration of radiotherapy (RT) and temozolomide (TMZ) to animal subjects, is monitored by bioluminescence imaging. Recurrent tumors were resected from brain then sequentially propagated as subcutaneous xenografts through three successive mouse hosts that received no treatment during tumor propagation. Subcutaneous tumor from the third mouse host was used for establishing intracranial tumors in a new series of mice that received the same treatments as in the initial experiment, in order to assess whether recurrent, treated tumors respond differently to the same treatments used in treating corresponding therapy naïve tumors. We observed no indication of tumor recurring from one cycle of RT + TMZ treatment as displaying resistance to the same treatments used in treating corresponding therapy naïve tumor. However, recurrent tumor that was subjected to two additional cycles of TMZ treatment, prior to resection from brain and subcutaneous propagation through 3 successive mice (107 days total subcutaneous propagation while receiving no treatment), displayed TMZ resistance when tested for response to the same treatments as corresponding naïve tumor. Median TMZ treatment survivals for mice intracranially engrafted with naïve, RT + 1 cycle TMZ treated, and RT + 3 cycles TMZ treated tumor cells were 80, 74, and 42 days, respectively, and survival results yielded log-rank p-values <0.001 for naïve and RT + 1 cycle TMZ comparisons against mice with RT + 3 cycle TMZ treated intracranial tumor. These results suggest that GBM PDX with stable TMZ resistance can be developed in vivo by repetitive, cyclical administration of TMZ to mice with intracranial tumor. Results from the multidimensional analysis of

such PDX should yield useful information for testing therapeutic hypotheses aimed at improving treatment outcomes for patients with recurrent GBM.

CSIG-19. DETECTION OF IDH1 R132H VIA FLOW CYTOMETRY IN INTRAOPERATIVE AND ARCHIVAL GLIOMA SPECIMENS

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Gliomas are the most common adult primary brain tumor and remain incurable. One critical challenge in the study of glioma is separating the biological effects of diverse cells that make up a tumor. Mass cytometry is a method used to simultaneously detect 37 or more epitopes per-cell on tens of thousands of cells isolated from patient tumor samples. This technology has been widely used in blood cancers and has recently been employed in solid tumors including glioma patient samples. However, a definitive protein marker that can distinguish glioma versus non-glioma stromal cells in flow cytometry does not exist. The isocitrate dehydrogenase 1 (IDH1) enzyme is mutated in approximately 80% of grade II and III gliomas and 10% of glioblastomas, with R132H as the most common mutation. Mutant IDH1 is found exclusively in cancerous cells and, when incorporated in flow cytometry experiments, positively identifies these cells in IDH-mutant tumors. We have developed a protocol to detect the IDH1 R132H antigen in fluorescence and mass cytometry. This protocol has been successfully combined with other staining and preparation steps to identify CD45-positive immune cells, CD31-positive endothelial cells, and IDH R132H-positive tumor cells in engineered cell lines, primary patient samples, and material extracted from paraffin blocks. These cell populations can be computationally separated for further analysis of protein expression, high dimensional phenotypes, signaling features, and cell abundance. Flow cytometry can be used to confidently identify a subset of cancer lineage cells and compare their protein phenotypes to other cells within the tumor as well as to other patient samples. This technique will ultimately provide a useful complement to other single-cell approaches and enable tracking of mutant IDH1 expression over the duration of functional assays such as drug response or engraftment.

CSIG-20. GBP2 ENHANCES GLIOBLASTOMA INVASION THROUGH STAT3/FIBRONECTION PATHWAY

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Glioblastoma multiforme (GBM) is the most common and deadliest form of brain tumor in adults. Guanylate-binding protein 2 (GBP2) is an interferon-inducible large GPTase with antimicrobial activities. However, its biological function in cancer remains largely unknown. Here we show that GBP2 expression is highly elevated in GBM tumor and cell lines, particularly those of the mesenchymal subtype. High GBP2 expression is associated with poor outcomes for GBM patients. GBP2 deregulation has no obvious effects on GBM cell proliferation in vitro. GBP2 overexpression significantly promotes GBM cell migration and invasion, and GBP2 silencing by RNA interference exhibits opposite effects. We further show that fibronectin (FN1) is dramatically induced by GBP2 expression at both mRNA and protein levels, and FN1 is essential for GBP2-promoted GBM invasive capacity. Inhibition of STAT3 pathway prevents GBP2-promoted FN1 induction and cell invasion. Consistently, GBP2 dramatically promotes GBM tumor growth and invasion in mice, and GBP2 expression significantly shortens the survival time of the mice with tumor. Together, these findings establish the role of GBP2/STAT3/FN1 signaling cascade in GBM invasion and suggest GBP2 may serve as a novel potential therapeutic target for inhibiting GBM invasion.

CSIG-21. THE ROLE OF miR-219a-2-3p AS A TUMOR SUPPRESSOR IN IDH1/2-WILD-TYPE GRADE II/III GLIOMAS

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IDH1/2 mutation status can accurately stratify distinct groups of lower-grade glioma (LGG) patients with *IDH1/2* non-mutant patients having significantly worse survival rates. A better understanding of the molecular alterations in *IDH1/2* mutant versus non-mutant patients may lead to new therapeutic options for the high-risk non-mutant population. We conducted a global microRNA (miRNA) expression analysis using the NanoString array to identify miRNAs differentially expressed in *IDH1/2* mutant versus non-mutant grade II/III glioma patients. Of the miRNAs differentially expressed, we performed additional validation experiments confirming *miR-219a-2-3p*, which provided strong rationale to characterize this miRNA in gliomas. *miR-219a-2-3p* was significantly upregulated 3.4-fold (FDR p = 0.026) in *IDH1/2* mutant versus non-mutant tumors (n=56) and was validated in the TCGA LGG cohort. Upon univariable Cox-regression analysis, *miR-219a-2-3p* was significantly associated with better overall survival (HR = 0.73 (0.55–0.96); p = 0.03). We hypothesized that *miR-219a-2-3p* may act as a tumor suppressor in *IDH1/2* non-mutant gliomas. Overexpression of *miR-219a-2-3p* in LN18 (*PTEN* wild-type) and U87 (*PTEN* mutated) glioma cell lines harboring wild-type *IDH1/2* resulted in a significant inhibition of cell proliferation, colony formation, and cell invasion. We also observed a significant radiosensitizing effect of *miR-219a-2-3p*. Using an *in silico* approach, we identified four putative targets of *miR-219a-2-3p*. Overexpression of *miR-219a-2-3p* significantly downregulated each target at the mRNA and protein levels. These targets have been shown to play a role in tumor progression by activating the PI3K-AKT and Ras-ERK pathways. Accordingly, we observed a significant decrease in AKT phosphorylation when *miR-219a-2-3p* was overexpressed. These data suggest that *miR-219a-2-3p* and/or its targets may be potential candidates to pursue as novel therapeutic options in *IDH1/2* wild-type gliomas. Currently, *in vivo* experiments for *miR-219a-2-3p* are in progress. FUNDING: R01CA108633, R01CA169368, RC2CA148190, U10CA180850-01(NCI), Brain Tumor Funders Collaborative Grant, and the Ohio State University Comprehensive Cancer Center (all to AC).

CSIG-22. RECONCILING TUMOR HETEROGENEITY IN GLIOBLASTOMA USING A PATHWAY-BASED APPROACH

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Despite efforts to gain a deeper understanding of its molecular architecture, glioblastoma (GBM) remains uniformly fatal. While genome-based molecular subtyping has revealed that GBMs may be parsed into several molecularly distinct categories, this insight has yielded little progress towards extending patient survival. In particular, the great phenotypic heterogeneity of GBM –both inter and intratumorally – has hindered therapeutic efforts. To this end, we interrogated tumor samples using a pathway-based approach to resolve tumoral heterogeneity. Gene set enrichment analysis (GSEA) was applied to gene expression data and used to provide an overview of each sample that can be then compared to other samples, to generate sample clusters based on their overall patterns of enrichment. The Cancer Genome Atlas (TCGA) samples were clustered using the canonical and oncogenic signatures and in both cases the clustering was distinct from the molecular subtype previously reported. Using principal component analysis (PCA) and other bioinformatics tools we extracted gene sets to further characterize the pathways contributing to each of these clusters. We generated gene lists comprised of the top common elements and Ingenuity pathway analysis exposed molecular targets that control critical pathways of each identified cluster. Similar analyses were completed in a gene expression database of patient-derived gliomasphere lines and in datasets that address intratumoral heterogeneity: 1) sorted stem cell populations compared with unsorted fractions and 2) lines derived from tumor cores, versus those derived from tumor periphery of the same patients. Validation studies are in progress, to assess whether molecules of interest may be targeted in lines from a particular cluster and to determine treatment responsiveness in terms of tumor cell cycle kinetics, proliferation, survival and self-renewal. Our studies relate inter- and intratumoral heterogeneity to critical cellular pathways dysregulated in GBM, with the ultimate goal of establishing a pipeline for patient- and tumor-specific precision medicine.

CSIG-23. miR-146a PREDICTS BETTER OVERALL SURVIVAL AND ACTS AS A TEMOZOLOMIDE (TMZ) SENSITIZER THROUGH TARGETING MULTIPLE SIGNALING PATHWAYS IN GLIOBLASTOMA

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BACKGROUND: Glioblastomas (GBMs) are the most aggressive primary brain tumors, with an average survival of less than 15 months. microRNAs (miRNAs) are emerging as promising, novel prognostic biomarkers in GBM. miRNA-146a acts as a tumor suppressor in different cancer types. This purpose of this study is to assess the prognostic value of miR-146a and investigate its role in tumor growth and therapeutic sensitivity and the underlying cellular and molecular mechanisms in GBM. METHODS: To determine the correlation between expression of miR-146a and overall survival (OS) of GBM patients, total RNAs were isolated from 268 FFPE GBM tumor samples (*IDH1/2* WT) for miR expression ana-

lysis (simultaneously) using the nCounter human miRNA v3a assay (NanoString Technologies), then, univariable and multivariable analyses were performed. Furthermore, functional studies were conducted to define the role of miR-146a in GBM tumorigenesis and therapeutic response and the underlying molecular mechanisms in established and patient-derived primary GBM cells. RESULTS: Univariable analyses demonstrated that miR-146a is correlated with better prognosis in GBM patients (HR=0.66, p=9.21E-05), which was independent of the promoter methylation of O^6-methylguanine-DNA methyltransferase by multivariable analyses (p<0.001). Interestingly, expression of miR-146a was downregulated in a majority of GBM cells compared with normal human astrocytes, as well as GBM tissues compared with paired non-tumor tissues. Functionally, overexpression of miR-146a significantly inhibited proliferation and invasion of both established and patient-derived primary GBM cells in vitro. Importantly, we also found that miR-146a overexpression increased the sensitivity of GBM cells to temozolomide (TMZ) and potentiated TMZ-induced apoptosis in vitro. While conducting downstream targeting and mechanistic studies, we found that overexpression of miR-146a significantly inhibited the NF-κB, AKT, and ERK pathways through targeting novel targets in GBM. CONCLUSION: Our data suggest that miR-146a predicts favorable prognosis for GBM patients and may serve as a therapeutic target in GBM.

CSIG-24. miR-575 IS ASSOCIATED WITH WORSE SURVIVAL OF GBM PATIENTS AND ACTS AS A NOVEL ONCOGENE THROUGH TARGETING p27/CDKN1B AND BLID/BRCC2

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Glioblastoma (GBM) patients currently face poor survival outcomes with an average survival period of less than 15 months, while only 3–5% of patients survive longer than 36 months. Although the mechanisms of tumorigenesis are still being elucidated, miRNAs are promising candidates to explore as novel and prognostic biomarkers in GBM. Here, we determined the correlation between miR-575 expression in GBM tumors and overall survival (OS) of patients and undertook functional studies to unfold the contribution of this miR to GBM tumorigenesis. Total RNAs were isolated from 268 FFPE GBM tumor samples and miR expression was assayed (simultaneously) using NanoString Technologies, and univariable and multivariable cox regression analyses were performed. Cell proliferation, colony formation, migration assay, qPCR, immunoblotting, and luciferase assay were conducted to define the function of miR-575 in GBM. Our survival analysis (n=268) show that miR-575 is associated with worse OS of GBM patients (HR=1.5, p-value=5.77E-05) by continuous UVA. miR-575 was also found to be significantly associated with OS, independent of age, gender, treatment, and KPS in a MVA (HR=1.208, p=0.012). Since miR-575 was found to be negatively associated with OS, we hypothesized that miR-575 overexpression would increase tumor progression. Our functional studies indicate that overexpression of miR-575 significantly increased the proliferation and motility of LN229 and U251 GBM cells *in vitro*. Consistent with the result found in LN229 and U251 cells, inhibition of miR-575 in U87 cells significantly decreased their proliferation and motility. Subsequent *in silico* and mechanistic studies identified *p27/CDKN1B* and *BLID/BRCC2* aspotential target genes of miR-575 in GBM. Overexpression of miR-575 in GBM cells reduced the expression of both *BLID* and *p27* at both mRNA and protein levels. miR-575 has prognostic value in GBM, with higher expression correlating with worse OS of patients, and contributes to GBM tumorigenesis by inhibiting expression of *p27* and *BLID*.

CSIG-25. EPIDERMAL GROWTH FACTOR RECEPTOR EXTRACELLULAR DOMAIN MISSENSE MUTATION A289V AS A DRIVER OF GLIOBLASTOMA INVASION AND PROLIFERATION

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Epidermal Growth Factor Receptor (EGFR) missense-mutations in glioblastoma (GBM) typically occur within the extracellular domain (ECD) and, to-date, have not shown a clinical impact. These ECD mutations, while not as common as wild-type amplification or EGFRvIII, do represent a distinct patient population in need of investigation. This study investigates the oncogenic effect of the most common EGFR ECD missense-mutations (R108K, A289D/T/V, and G598V) in a retrospective cohort of 260 *de novo* GBM patients from the University of Pennsylvania. Extrapolation of each individual missense-mutation revealed a significant reduction in patient survival for the $EGFR^{A289D/T/V}$ mutants (p=0.028). This effect was confirmed with a follow-up cohort of 111 patients. The Cancer Imaging Phenomics Toolkit (CaPTk – www.cbica.upenn.edu/captk) was used to extract 2104 quantitative imaging phenomic (QIP) features, from multiparametric magnetic resonance imaging, across the various tumor sub-regions comprising the enhancing and non-enhancing tumor core, as well as the peritumoral edematous/invaded tissue. Multivariate machine learning techniques integrated these features, revealing radiographic signatures suggestive of increased invasion and proliferation for patients bearing the A289 mutation. The underlying mechanism of $EGFR^{A289V}$ in tumor growth was examined using engineered U87 glioma cells and patient-derived HK281 glioma spheres simulating expression of wild-type-EGFR or $EGFR^{A289V}$. Corroborating our findings in patients, mice bearing intracranial tumors with $EGFR^{A289V}$ mutations revealed significantly worse survival accompanied by highly invasive tumors. Cells expressing $EGFR^{A289V}$ yielded a highly active EGFR/ERK signaling pathway, resulting in increased expression and functionality of the matrix metalloproteinase MMP1, which can be attenuated by the use of ERK pathway inhibitors. Collectively, the findings of this study highlight a highly invasive and proliferative phenotype associated with the $EGFR^{A289V}$ missense-mutation. Moreover, given the tumor-specific and extracellular nature of the mutation, we postulate that it may be amenable to targeted therapy. Key data presented is from a manuscript with preliminary acceptance to Cancer Cell. *equal contribution

CSIG-26. MOLECULAR DETERMINANTS OF SENSITIVITY TO PI3K/AKT/mTOR PATHWAY INHIBITION IN GLIOBLASTOMA

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Molecular genetic aberrations in the phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway are common in human cancers including glioblastoma, yet, novel therapeutic approaches targeting this pathway in glioblastoma have had limited success to date. Here we analyzed the molecular mechanisms determining sensitivity to PI3K/AKT/mTOR inhibition using gene silencing or pharmacological inhibition by assessing target inhibition, modulation of down-stream signaling pathways, viability, proliferation, and clonogenicity (sphere formation) in human *isocitrate dehydrogenase (IDH)* wild-type long-term cell (LTC) lines and glioma-initiating cells (GIC). Glioma cells including GIC in particular were universally sensitive to growth inhibition induced by PQR309, a novel, dual pan-PI3K/AKT/mTOR antagonist *in vitro*. Cells exhibited profound growth arrest, but little apoptotic or necrotic cell death as confirmed by electron microscopy; yet, there was evidence of senescence. Cell lines with high basal levels of phosphorylated (active) AKT, low levels of phosphorylated (inactive) protein translation repressor eukaryotic initiation factor (eIF) 4E-binding protein 1 (p4E-BP1), and high levels of *Ser9*-phosphorylated (inactive) glycogen synthase kinase 3 beta (pGSK3β) were more sensitive to PQR309. Accordingly, PQR309 acted synergistically in combination with AKT inhibitors to inhibit clonogenicity or spherogenicity *in vitro*, indicating that persistent AKT activity may represent an escape mechanism from PI3K/AKT/mTOR-targeted therapy. *In vivo* studies confirmed the anti-glioma activity of PQR309 alone or in combination with AKT inhibition in the orthotopic LN-229 glioma model. Altogether, these data may help to stratify or enrich for patients likely to benefit from PI3K/AKT/mTOR inhibition in future clinical trials of targeted therapy in glioblastoma.

CSIG-27. DIFFERENTIAL ELEVATION OF TERT ACTIVITY AND SENSITIVITY TO TEMOZOLOMIDE BY TYPE OF TERT MUTATION IN MGMT PROMOTER-METHYLATED GLIOBLASTOMA

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BACKGROUND: Benefit from temozolomide chemotherapy in glioblastoma is essentially limited to patients with tumors that exhibit O^6-methylguanine DNA methyltransferase (MGMT) promoter methylation. Recent retrospective clinical analyses indicate that the impact of the *MGMT* status on chemosensitivity may be modulated by telomerase reverse transcriptase (TERT) promoter mutations. These commonly affect two regions of the TERT promoter, C228T and C250T. METHODS: TERT promoter mutation status and TERT activity were determined and correlated with sensitivity to irradiation or temozolomide in long term and glioma-initiating cell lines. TERT status alterations were induced using sh-mediated gene TERT silencing or wildtype TERT overexpression. TERT mutation and MGMT promoter methylation status were also determined in a clinical patient cohort from the German Glioma Network. RESULTS: C228T-mutant glioma cell lines (n=8) show higher TERT mRNA expression (mean 0.046 ± 0.012 vs 0.012 ± 0.004 arbitrary units, p=0.049) and higher TERT catalytic activity (mean 122 ± 16 vs 53 ± 11 arbitrary units, p=0.022) than C250T-mutant glioma cell lines (n=5). C228T-mutant glioma cell lines are also more sensitive to irradiation (mean ED90 4.6 ± 0.7 versus 7.1 ± 0.8 Gy, p=0.039) or temozolomide (mean EC50 101.6 ± 58.5 versus 295.2 ± 53.8 µM, p=0.045) *in vitro*. Targeted alterations of TERT status affect sensitivity to irradiation or temozolomide: TERT gene silencing confers protection whereas TERT overexpression confers sensitization. Consistent with these preclinical observations, patients with C228T TERT mutation and MGMT promoter methylation may derive more benefit from temozolomide chemoradiotherapy (median overall survival 26.5 months, 95% CI 20.3–32.7) than patients with the C250T mutation (median overall survival 16.2 months, 95% CI 8.5–23.8) or patients without TERT mutation (median overall survival 23.7 months, 95% CI 18.7–28.8). CONCLUSIONS: These results confirm a link between TERT promoter status and sensitivity to irradiation and temozolomide and illustrate how TERT and MGMT might interact to determine outcome in human glioblastoma. They allow to design future targeted interventions focusing on TERT and MGMT to improve the treatment of glioblastoma.

CSIG-28. LONG NONCODING RNA MIR22 HOST GENE-DERIVED miR-22-3p AND miR-22-5p PROMOTE PROLIFERATION, INVASION AND SELF-RENEWAL IN HUMAN GLIOMAS VIA Wnt/-CATENIN SIGNALING

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BACKGROUND: Long noncoding RNAs (lncRNAs) are essential transcripts with critical roles in tumor initiation and malignant progression. In the present study, through analysis of whole-transcriptome profiles, we showed that MIR22 host gene (MIR22HG), the primary microRNA of miR-22, ranked among the most overexpressed lncRNAs in glioblastoma (GBM). Herein, our study aims to elucidate the biological functions of MIR22HG and therapeutic potency of MIR22HG targeted inhibition in GBM. METHODS: We characterized the expression pattern of MIR22HG/miR-22 by bioinformatics analysis and by in situ hybridization (ISH) in GBMs. We further determined whether genetic targeting of MIR22HG affected oncogenic functions of primary GBM cells *in vitro* and *in vivo*. A co-culture model where brain organoids are co-cultured with GBM spheroids were utilized for the invasion assessment. Finally, based on the three-dimensional structure of the pre-miR-22, we performed virtual screening strategy to disclose a novel inhibitor of the MIR22HG/miR-22 axis. RESULTS: We showed that MIR22HG/miR-22 was highly expressed in GBM, especially in isocitrate dehydrogenase 1/2 (IDH1/2) wild-type tumors. Specially, MIR22HG/miR-22 was preferentially expressed in glioblastoma stem cells (GSCs) compared to normal neural stem cell (NSCs). Analysis of intra-tumoral transcriptional heterogeneity showed that MIR22HG was higher in the infiltrating region compared to the tumor center. In functional assays, silencing MIR22HG attenuated aggressive phenotypes in GBM cells, including invasion, self-renewal and *in vivo* tumor growth via suppressing the production of miR-22-3p and miR-22-5p. Using a luciferase reporter assay, we further confirmed that two inhibitors of the Wnt/β-catenin pathway, SFRP2 and PCDH15, were direct targets of miR-22-3p and miR-22-5p. Finally, we identified a specific inhibitor termed AC1L6JTK, which blocks the ability of Dicer to process pre-miR-22 to mature miR-22, thus exerting therapeutic efficacy on GBMs. CONCLUSIONS: Our findings show that the MIR22HG/miR-22 axis is a novel biomarker as well as a therapeutic target in GBM.

CSIG-29. THE DUAL PI3K/mTOR-PATHWAY INHIBITOR GDC-0084 ACHIEVES ANTITUMOR ACTIVITY IN BREAST CANCER BRAIN METASTASES IN VITRO AND IN VIVO

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Breast cancer is the second most common primary tumor leading to brain metastases in adult cancer patients. Previous studies have shown that the PI3K/AKT/mTOR pathway in breast cancer brain metastasis is activated in up to 70% of analyzed tumors. However, there are no approved agents targeting this pathway in breast cancer brain metastases at present. GDC-0084 is a dual brain penetrant PI3K/mTOR-inhibitor that has already demonstrated promising response rates in a preclinical glioblastoma model. The aim of this study was to analyze the efficacy of this compound in a breast cancer brain metastases model in vitro and in vivo using PIK3CA-mutant and PIK3CA-wildtype cell lines. In vitro methods included cell viability, apoptosis, growth inhibition assays, cell cycle analysis, Western blots and immunohistochemistry. In vivo, the effect of GDC-0084 was evaluated in an orthotopic intracranial mouse model with bioluminescent imaging. GDC-0084 induced apoptosis in PIK3CA-mutant breast cancer brain metastases cell lines and growth inhibition in PIK3CA-wildtype cell lines in vitro and markedly inhibited tumor growth of PIK3CA-mutant cell lines in vivo. The results of this study highlight the importance of brain-penetrant agents targeting the PI3K/AKT/mTOR-pathway and suggest that GDC-0084 might be a promising treatment option for breast cancer brain metastases patients in the future.

CSIG-30. DEUBIQUITINATING ENZYME (USP5) COOPERATE WITH RNA BINDING PROTEIN (hnRNPA1) DURING GLIOMA PROGRESSION, BRING THERAPEUTIC INSIGHTS

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Glioma is a challenging disease with median survival less than a year. Disease reoccurrence where RNA binding proteins are potentially involved in modulating the whole cascade of event. Flexibility in alternative splicing variants formation are the major events, where key regulators (RBP's) are under the control of deubiquitinating enzymes. Expression analysis of USP5 were evaluated in between Grade II and Grade IV Gliomas immunohistochemistry. USP5 expression were elevated in grade IV GBMs, whereas Grade II lacks its expression. USP5 expression were positively correlated with hnRNPA1(RBP). Western blotting experiments were performed in USP5 knock down glioma cell line shows downregulation of hnRNPA1. Similarly, interaction between USP5 and hnRNPA1 were performed, indicating a positive cooperation between USP5 and hnRNPA1. Ubiquitination and deubiquitination profiling of hnRNPA1 from grade to grade basis will help us in evaluating the disease outcome. Screening of drugs against USP5 activity along with expression studies of RNA binding proteins, would be a best strategy, to evaluate the therapeutic efficacy.

CSIG-31. MUTATION OF PIM1 GENE IN PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA INHIBITS CELL DEATH THROUGH CHANGE IN SUBCELLULAR LOCALIZATION OF PIM-1 AND INCREASE OF BAD PHOSPHORYLATION

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BACKGROUND: In a study of Next Generation Sequencing in primary central nervous system lymphoma (PCNSL), we have previously reported

several mutations of high frequency, in comparison with systemic diffuse large B cell lymphoma (DLBCL)s. Consequences of these specific mutations in PCNSL are unknown. In this study, we have analyzed the functional consequence of mutations in the *PIM1* gene, observed in 100% of PCNSL patients, which encodes a serine/threonine kinase and is known to drive tumorigenesis in several malignancies. METHODS: Four most frequent mutations of *PIM1* in PCNSL, *S77N*, *K115N*, *P216S*, *L275F*, were chosen from our previous study, and each mutant was generated by site directed mutagenesis in *PIM1* cDNA cloned in an expression vector. Resulting vectors were transiently transfected into human cancer cell lines. Cell death of the cells expressing each mutant was evaluated by dye-exclusion method under treatment of chemotherapeutic agents. Alteration of molecular signaling was evaluated by immunoblotting. RESULTS: Among the four mutants, increased phosphorylation of BCL-2 associated death promoter (BAD) at Ser112, which is a phosphorylation target of Pim-1, was observed by expression of *K115N* mutant compared with wild type *PIM1* in Nagai and Hela cells expressing endogeneous BAD. Decreased cell death under campthotethin treatment was also observed in *K115N* mutant expressing Nagai cells compared with wild type *PIM1*-expressed cells. Moreover, we observed a significant shift in subcellular localization of Pim-1 carrying *K115N* mutant; from the nucleus, main sublocalization for wild type Pim-1, into the cytosol determined by immunocytochemistry and immunoblotting of nuclear and cytosolic fraction of the cells. DISCUSSION: It is suggested that *PIM1 K115N* mutant may drive chemoresistance through increased BAD phosphorylation that suppresses cell death compared with wild-type *PIM1* through modification of its subcellular localization.

CSIG-32. DUAL PI3K/Akt INHIBITION TO OVERCOME THE P-gp/BCRP DRUG EFFLUX SYSTEM FOR IMPROVED DRUG DELIVERY IN GLIOBLASTOMA THERAPY

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The blood-brain barrier is a primary obstacle for effective anticancer drug therapy of patients with glioblastoma multiforme (GBM). On a molecular level, failure of anticancer drug treatment is largely due to the blood-brain barrier efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). P-gp and BCRP (P-gp/BCRP) work together to restrict anticancer drugs from crossing the barrier and from entering the brain to reach tumor targets. We found that PI3K/Akt regulates P-gp/BCRP in brain capillaries of the rodent and human blood-brain barrier. Our *in vivo* data show that combination treatment with LY294002 (PI3K inhibitor) and triciribine (Akt inhibitor) downregulates P-gp and BCRP protein expression and transport activity in brain capillaries. We also have evidence from brain capillaries isolated from GBM mice and GBM patients showing that GBM induces P-gp/BCRP overexpression in capillaries in the brain hemisphere that is *contralateral* to the primary tumor. These findings indicate that P-gp/BCRP overexpression in brain capillaries protects invasive tumor cells that are scattered throughout the brain from being targeted by anticancer drugs. To overcome this obstacle, we are currently developing a novel therapeutic strategy by targeting PI3K/Akt to transiently decrease P-gp/BCRP expression and activity, thus, creating a "window-in-time" during which anticancer drugs can enter the brain.

CSIG-33. ONCOLYTIC HERPES VIRUS TREATMENT INDUCED NOTCH SIGNALING VIA HSV-1 microRNA H16 AND IT INVOLVED IN TREATMENT RESISTANCE

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INTRODUCTION: Glioblastomas (GBMs) are resistant to traditional therapies. Thus, the development of novel treatment strategies is urgently needed. NOTCH signaling is activated in GBM and important for mediating proliferation, angiogenesis, and resistance to therapy. In this study, we uncovered the mechanism by which oncolytic herpes simplex viruse-1 (oHSV) therapy induced NOTCH activation in the tumor microenvironment (TME). We also investigated the therapeutic benefit of combining oHSV therapy with Gamma-secretase inhibitor (GSI), a NOTCH inhibitor. METHODS: Real time Q-PCR, NOTCH reporter assay, and bioluminescence mice imaging were used to test oHSV-induced NOTCH activation. Screening of HSV-1-encoded microRNAs (HSVmiRs) and genes were performed to identify the mechanism which regulates the NOTCH signaling. Intracranial glioma-bearing xenografts were used to evaluate the anti-tumor efficacy of combination of GSI and oHSV. RESULTS: oHSV infection induced gene expression of NOTCH ligands resulting in activation of NOTCH signaling in adjacent cells in primary GBM derived cells. This was mediated by HSV encoded miRH16. HSVmiRH16 targets and suppresses Factor Inhibitor Hif1-1 (FIH-1) expression which is known to inhibit NOTCH activation. Consistent with this both overexpression of HSVmiRH16 and knockdown of FIH-1 significantly induced NOTCH signaling. Treatment of mice bearing intracranial glioma with GSI and oHSV therapy significantly enhanced survival compared to that with monotherapy. CONCLUSION: We identified oHSV-induced NOTCH signaling activation, via HSV encoded miRH16. We further find that GSI treatment in combination with oHSV therapy demonstrated improved efficacy. To our knowledge this is the first report investigating NOTCH signaling in conjunction with oHSV therapy.

CSIG-34. PI3 KINASE PATHWAY ACTIVATION PROMOTES MALIGNANT PROGRESSION IN OLIGODENDROGLIAL TUMORS

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Oligodendroglioma (OD) is a subtype of adult diffuse glioma defined by IDH1/2 gene mutation and co-deletion of chromosomal arms 1p and 19q. Although prognosis in OD tumors is initially relatively favorable, the majority of OD develop outgrowth of a subclone that has undergone malignant transformation. Modeling the molecular mechanisms of this tumor progression is crucial to identify therapeutic targets for malignant disease. However, there are few available patient-derived OD xenograft models, which limit preclinical investigations. Here, we present novel patient derived anaplastic oligodendroglioma (AOD) xenograft models. In a panel of OD at different stages of disease, we harvested two distinct cell samples: those with and without PIK3CA mutation. From the tumor that subsequently rapidly progressed and had a *PIK3CA* mutation, we established a xenograft model that was lethal to the mouse and retained the *PIK3CA* mutation. In contrast, xenograft did not form from the other tumor that was clinically stable after resection and had wild-type *PIK3CA*. We confirmed AOD phenotype and the presence of IDH1 mutation and 1p/19q co-deletion in xenograft tissue, indicating successful capture of these signature OD genetic alterations. We also tested to see if PI3K/AKT/mTOR gene mutation could induce patient-derived OD xenograft formation. In our attempts to establish xenograft models, the presence of activating mutations in PI3K/AKT/mTOR pathway was consistently associated with successful xenograft establishment. OD/AOD tumors that did not form xenograft did not have mutation in the PI3K/AKT/mTOR pathway Importantly, we found progressive tumor cells that harbor mutant *PIK3CA* were vulnerable to alkylating agents and PIK/AKT/mTOR pathway inhibitors. These findings suggest there is a critical role of PI3K/AKT/mTOR pathway activation in driving progression and xenograft formation in oligodendroglial tumors. Our xenograft models will facilitate dissection of the mechanism of malignant transformation, contributing to the identification of optimal therapeutic strategies for patients with oligodendroglial tumors

CSIG-35. MST4 PHOSPHORYLATION OF ATG4B REGULATES AUTOPHAGIC ACTIVITY, TUMORIGENICITY, AND RADIORESISTANCE IN GLIOBLASTOMA

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Autophagy is a conserved catabolic process that maintains homeostasis by regulating the energy balance of the cell. Cancer cells use autophagy to re-

move damaged organelles and aggregated proteins, and to recycle nutrients in high demand to support tumor growth. Radiation therapy (RT) and temozolomide (TMZ), are front-line treatments for glioblastoma (GBM), the common and most malignant brain tumors in humans. However, RT and TMZ are known activating the autophagic response in tumor cells, which protects GBM cells from therapy-induced cell death. Thus, improved understanding of mechanisms regulating autophagy could reveal targets for selective and specific inhibition, which would enhance the anti-tumor activity of RT and TMZ while reducing toxic effects of treatment. In this study, we determined the roles of MST4, a less known protein serine/threonine kinase in its cellular functions in regulation of GBM tumorigenicity and therapy responses through activating autophagic activities. By using proteomic, biochemical and genetic approaches, we identified ATG4B as a novel substrate of MST4. ATG4B is a key regulator that facilitates autophagic process through reversible modification of ATG8/LC3. MST4 phosphorylates ATG4B at serine residue 383, which stimulates ATG4B enzymatic activity towards LC3, increasing autophagic flux. Inhibition of MST4 or ATG4B activities suppresses autophagic activities and tumorigenicity of patient-derived glioma stem cells (GSCs) in vitro and in the brain of mice. Furthermore, RT induces MST4 expression, ATG4B phosphorylation and autophagic activity. Inhibiting ATG4B by using a novel inhibitor NSC185058 in combination with RT in treating mice with intracranial GBM tumor xenografts markedly slows tumor growth and provides significant survival benefit to animal subjects. This study not only describes a novel regulatory mechanism by which the MST4-ATG4B axis accelerates autophagic process, regulates GBM tumorigenicity, and responses to RT, but also explores imminent clinical utility of combination of ATG4B inhibition with RT to suppress orthotopic GBM tumor xenografts.

CSIG-36. INVOLVEMENT OF microRNAs 221/222-3p IN THE REGULATION OF PROGRAMMED CELL DEATH 10 (PDCD10) GENE IN GLIOBLASTOMA

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INTRODUCTION: FAT1 gene is localized at chromosome 4q35.2 encoding a 506KDa. Here in our study we are characterizing the role of FAT1 in primary brain tumors. MiR-221-3p/222-3p reported to have oncogenic role and targets tumor suppressors (e.g. PDCD10, PTEN, PUMA etc.) in many cancers including GBM. Here, we have analyzed the role of FAT1 gene in the regulation of miRNAs in GBM. METHODOLOGY: In-silico analysis of miR targets was done by target prediction software miRDB, TargetScan, miRTarBase. FAT1 knockdown was done using FAT1 specific siRNA and mRNA expression analysis done by gene specific primers and for miR-221/222-3p using LNA-primers in GBM cell lines (U87MG, U373MG, A172 and LN229). Expression and Spearman correlation analysis of FAT1 and miR-221-3p was done in GBM tumor samples (n=30). RESULTS: We have observed increased expression of FAT1 and miRNAs (miR221-3p/miR222-3p) in GBM cell lines (U87MG, U373MG, A172 & LN229). On FAT1 knockdown, by siFAT1 we observed significantly reduced expression of miR-221/222-3p. In-silico analysis identified, TIMP3, PDCD10, PUMA and PTEN as potential targets of miR-221/222-3p. Furthermore, FAT1 knocked-down cells showed significantly augmented expression of PDCD10 in all studied glioma cell lines. In order to validate our in-vitro observation and its clinical relevance, we have done expression and correlation study in GBM tumor samples. We observed significant positive spearman correlation between FAT1 and miR-221-3p ($r=0.5669$, $p\leq0.0011$) and negative correlation of FAT1 with PDCD10 ($r= -0.3492$, $p\leq0.0585$),) and miR-221-3p with PDCD10 ($r=0.526$, $p\leq0.0028$). These results suggest that FAT1 expression positively regulates the expression of miR-221-3p leading to downregulation of miR 221-3p target (PDCD10) in GBM cell lines and GBM tumors. CONCLUSION: Taken together our in-vitro and GBM tumor data for the first time suggesting FAT1 to be a novel molecule regulating the expression of miRNA in GBM and FAT1 may emerge as a target for therapeutic intervention.

CSIG-37. FOXR2 STABILIZES MYC AND ACTIVATES FAK/SRC SIGNALING IN A DUAL MECHANISM TO PROMOTE TRANSFORMATION IN NEURAL PROGENITOR CELLS

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Medulloblastoma and central nervous system primitive neuroectodermal tumors (CNS-PNETs) are aggressive, poorly differentiated brain tumors that primarily affect children. Current treatment strategies with severe long-term treatment-related side effects and poor survival rates warrant further study into therapies with increased efficacy and lower cost to the patient. More targeted therapy represents a route to better treatments, but a barrier to identifying novel targets is a lack of animal models. We created a mouse model that developed medulloblastoma or CNS-PNET using *Sleeping Beauty* (*SB*) mutagenesis of neural progenitor cells (Nestin+). *SB*-induced tumors resembled human medulloblastoma and CNS-PNET histology. Additionally, we used RNA-Sequencing to determine that they most closely resemble human SHH, group 3, and group 4 medulloblastoma and a subgroup of CNS-PNET with *FOXR2* activation (CNS NB-*FOXR2*).Using both DNA and RNA analysis, we identified over 100 genes as candidate drivers in medulloblastoma and/or CNS-PNET. FOXR2 was identified as a proto-oncogene, with increased expression in *SB*-induced mouse tumors. FOXR2 drives colony formation in soft agar and tumor formation *in vivo* when overexpressed in a mouse neural progenitor cell line. We found that FOXR2 binds N-MYC and increases C-MYC stability in 2 neural cell lines. We also found a novel role for FOXR2 in activating the FAK/SRC signaling pathway. Increased FOXR2 drove FAK/SRC activation, in a MYC interaction-independent manner, and *FOXR2* KO decreased FAK/SRC activation. Interestingly, increased FOXR2 expression conveyed resistance to a SRC family kinase inhibitor (Dasatinib) in a MYC-dependent manner, indicating overlap between these two apparently distinct effects. Further studies into the mechanism of FOXR2-driven tumorigenesis may provide a novel route for therapy in treating patients with medulloblastoma and CNS-PNET with high FOXR2 levels.

CSIG-38. ROBO2 SIGNALING IN INVASION OF GLIOBLASTOMA

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Glioblastoma accounts for 54.3% of newly diagnosed glioma in the United States each year. Rapid growth and invasion confers a devastating phenotype with very limited treatment options at recurrence. Numerous studies of the molecular alterations in GBM have been conducted but few have completely characterized the invasiveness characteristic of GBM. The roundabout (Robo) family of transmembrane receptors and their Slit protein ligands, have been demonstrated to be involved in neuronal migration and outgrowth during CNS development and implicated in various cancers. We hypothesize that Slit-Robo pathway may be involved in GBM cell invasion. We reviewed numerous databases and analyzed protein and RNA from GBM samples. Analysis of the NCI Rembrandt database indicated poorer survival among glioma patients with a greater than two-fold overexpression of the Robo2 gene (17.1 months versus 37.4 months; p=1.42E-4). GBM tumor was identified prospectively on pre-operative MRI and sampled using stereotactic image-guided resection from various regions within the tumor. In tissue samples from 6 GBM and 3 control patients, indicted a 2.5 fold difference in the expression of Robo2 protein. Quantitative PCR and Western blot confirmed a 101- fold increase in mRNA expression. Knockdown of the mRNA using siRNA directed against Robo2 in U87 cells resulted in survival advantage (65.5 days versus 52.5 days; p=0.011). These data suggest a complex and heterogenous tumor microenvironment and implicates Robo axis signaling in GBM as a potential therapeutic target.

CSIG-39. HIV-1 ENVELOPE PROTEIN GP120 PROMOTES ACTIVATION OF PROTEIN SYNTHESIS IN GLIOMAS THROUGH THE ERK AND AKT SIGNALING

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Patients infected with human immunodeficiency virus (HIV-1) are more prone to developing cancers, including glioblastomas (GBMs). The median survival for GBM patients with HIV is significantly shorter than for HIV-negative GBM patients, despite the fact that they receive the same treatments. This difference indicates that HIV infection is associated with more aggressive tumor behavior and with treatment resistance. Earlier we demonstrated that gp120, a main glycoprotein in the HIV shell, stimulates glycolysis and protein synthesis in glioma cells. The purpose of this study was to evaluate the underlying gp120 dependent signaling mechanism in glioma cell. Using MAPK kinase antibody array and western blot assays we have identified the activation of MAP kinase and Akt/mTOR pathways in U87, A172, and primary glioma cells treated with gp120 (100 ng/ml) for 5 consequent days. Specifically, up regulation of pMEK1/2(Ser217/221), pERK(Thr202/Tyr224), pP90RSK(Thr359), pmTOR(S2448), pAkt(pS473), and pGSK3b(pS9) have been identified. These data coincide with previously obtained results showing that glioma cells treated with gp120 exhibit higher protein synthesis and proliferation rates compared to un-treated glioma cells. The use of pharmacological inhibitors of PI3K/Akt and ERK signaling reversed the stimulatory effect of gp120 on global protein synthesis, as

revealed by flow cytometry analysis. Overall, we demonstrated that gp120 triggers activation of ERK and Akt signaling in glioma cells, resulting in increased protein synthesis and cell growth. This research was made possible by NIH grant number 1SC1GM122691 and Puerto Rico Science, Technology, and Research Trust grant 2016-00157.

CSIG-40. HETEROZYGOUS IDH1$^{R132H/WT}$ CREATED BY "SINGLE BASE EDITING" INHIBITS HUMAN ASTROGLIAL CELL GROWTH AND PROMOTES CELL MIGRATION

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Mutations in the isocitrate dehydrogenase 1 (*IDH1*) gene have been identified in a number of cancer types, including brain cancer. The Cancer Genome Atlas project has revealed that *IDH1* mutations occur in 70–80% of grade II and grade III gliomas. Until recently, most of the functional studies of *IDH1* mutations in cellular models have been conducted in overexpression systems with the IDH1 wild type background. In this study, we employed a modified CRISPR/Cas9 genome editing technique called "single base editing", and efficiently introduced heterozygous IDH1 R132H mutation (*IDH1R132H/WT*) in human astroglial cells. Global DNA methylation profiling revealed hypermethylation as well as hypomethylation induced by *IDH1R132H/WT*. Global gene expression analysis identified molecular targets and pathways altered by *IDH1R132H/WT*, including cell proliferation, extracellular matrix, and cell migration. Our phenotype analysis indicated that compared with IDH1 wild type cells, *IDH1R132H/WT* promoted cell migration by up-regulating integrin b4 (ITGB4), and significantly inhibited cell proliferation. All these genotype and phenotype changes were reversed by mutant IDH1 inhibitor AGI-5198. Using our mutated *IDH1* models generated by genome editing, we identified novel molecular targets of *IDH1R132H/WT*, namely Yes-associated protein (YAP) and its downstream signaling pathway Notch, to mediate the cell growth-inhibiting effect of *IDH1R132H/WT*. In summary, the "single base editing" strategy has successfully created heterozygous *IDH1* R132H mutation that recapitulates the naturally occurring IDH1 mutation. Our isogenic cellular systems that differ in a single nucleotide in one allele of the *IDH1* gene provide a valuable model for novel discoveries of *IDH1R132H/WT*-driven biological events.

CSIG-41. ONCOGENE ADDICTION SWITCH FROM NOTCH TO PI3K REQUIRE SIMULTANEOUS TARGETING OF DUAL PATHWAY INHIBITION IN GLIOBLASTOMA

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Notch signaling pathway regulates normal stem cells in the brain and glioma stem cells (GSCs). However, blocking the proteolytic activation of NOTCH with γ-secretase inhibitors (GSIs) fails to alter the growth of some GSCs as GSIs seem to be active in only a fraction of GSCs lines with constitutive NOTCH activity. Here we report loss of PTEN as a critical event leading to resistance to NOTCH inhibition, which causes the transfer of "oncogene addiction" from the NOTCH to the phosphoinositol-3 kinase (PI3K) pathway. We investigated the effects of Notch inhibition in GSC using GSI. Drug cytotoxicity test on 16 GSCs show differential growth response to GSI stratifying GSCs into two groups: responders vs non-responders. Active Notch signaling seems to be important features for the GSC as Notch inhibition only affected GSCs defined as having increased Notch activity. However in the responder group GSCs with the PTEN mutation seems to be less sensitive to GSI treatment. Here we show that NOTCH regulates the expression of PTEN and the activity of the PI3K signaling pathway in GSCs since treatment with GSI attenuated Notch signaling and increases PTEN expression. NOTCH regulates PTEN expression via Hes-1 as knockdown of either Notch or Hes1 led to increase expression of PTEN.This novel observation suggests the need to simultaneous inhibition of both pathways as a means to improve therapeutic efficacy in human glioblastoma.

CSIG-42. HIGH THROUGHPUT KINOME AND TRANSCRIPTOME ANALYSES REVEAL NOVEL THERAPEUTIC TARGETS IN NF2-DEFICIENT MENINGIOMA

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Meningiomas (MN), the most common adult primary intracranial tumor, arise from the arachnoid/meninges and are non-responsive to chemotherapies with a high recurrence rate despite surgery, necessitating effective non-invasive therapies. Our previous work showed that *NF2* loss activates mechanistic target of rapamycin complex 1 (mTORC1) and mTORC2 signaling, which led to past NF2 clinical trials using rapalogs (RAD001/everolimus), and current meningioma clinical trials with dual mTORC1/mTORC2 inhibitor (mTORi) AZD2014. To understand additional dysregulated, potentially druggable pathways, we undertook an 'omics approach of large-scale kinomics and RNA-sequencing employing CRISPR-modified human arachnoidal cells (ACs), *NF2*-expressing vs *NF2*-null. In *NF2*-null ACs, several kinases were elevated including erythropoietin-producing hepatocellular (EPH)-receptor tyrosine kinase (RTK) family members, Src family kinase (SFK) members, and c-KIT, all targets of dasatinib. *In vitro* treatment of MN cells using mTORi (AZD2014 or INK128) and dasatinib enhanced growth inhibition upon combination mTORi+dasatinib. *In vivo* treatment of an orthotopic mouse MN model showed moderate response to dasatinib with stronger response using INK128 or INK128+dasatinib (e-published in Neuro-Oncology). Our transcriptomic data also revealed increased expression of several ligands/growth factors, particularly NRG1/neuregulin. Expanding these results, we have confirmed increased expression of NRG1 in human *NF2*-null ACs. We also find *NF2*-null ACs secrete NRG1, and in conditioned-media experiments we observe stimulation of ErbB3, EPHA2 and mTOR pathways, suggesting an autocrine signaling mechanism. *NF2*-null AC or MN cells, when stimulated with exogenous NRG1, show enhanced activation of mTOR and EPH pathways besides ErbB3 signaling. Further, lapatinib (multi-ErbB inhibitor) but not erlotinib (EGFR inhibitor) attenuates the NRG1-stimulated activation of ErbB3, EPHA2 and mTOR, suggesting that NRG1-induced activation is EGFR-independent. Taken together, our results support a mechanistic link where NF2 loss increases NRG1/ErbB signaling to EPH/SFK and mTOR pathways, which may be a critical driver of tumorigenesis, thus providing a therapeutic opportunity to co-target these pathways in *NF2*-deficient meningiomas.

CSIG-43. THE TYROSINE PHOSPHATASE PTPN12/PTP-PEST REGULATES PHOSPHORYLATION-DEPENDENT UBIQUITINATION AND STABILITY OF FOCAL ADHESION SUBSTRATES IN INVASIVE GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is an invasive brain cancer with tumor cells that disperse from the primary mass escaping surgical resection, displaying resistance to chemotherapy and radiation, and invariably giving rise to lethal recurrent lesions. Targeted therapies such as the anti-vascular endothelial growth factor (VEGF) blocking antibody bevacizumab have yielded disappointing results in GBM clinical trials, with no improvements in overall patient survival. Many patients treated with bevacizumab develop acquired resistance leading to lethal recurrent lesions associated with robust tumor cell invasion. While a great deal is known about genes and pathways that promote GBM proliferation and neovascularization, relatively little is understood about mechanisms that drive GBM cell invasion during progression or following anti-angiogenic therapy. Here, we report that PTP-PEST, a cytoplasmic protein tyrosine phosphatase encoded by the PTPN12 gene, controls GBM cell invasion by physically bridging the focal adhesion protein Crk-associated substrate (Cas) to valosin containing protein (Vcp), an ATP-dependent protein segregase that selectively extracts ubiquitinated proteins from multiprotein complexes and targets them for degradation via the ubiquitin proteasome system. Both Cas and Vcp are substrates for PTP-PEST, with the phosphorylation status of tyrosine 805 (Y805) in Vcp impacting affinity for Cas in focal adhesions and controlling ubiquitination levels and protein stability. Perturbing PTP-PEST-mediated phosphorylation of Cas and Vcp led to alterations in GBM cell invasive growth in vitro and in pre-clinical mouse models generated with GBM stem cells. Furthermore, acquired resistance to bevacizumab correlates with reduced expression of PTP-PEST in invasive GBM cells. Collectively, these data reveal a novel regulatory mechanism involving PTP-PEST, Vcp, and Cas that dynamically balances phosphorylation-dependent ubiquitination of key focal proteins involved in GBM cell invasion.

CSIG-44. TREATMENT WITH THE CASEIN KINASE 2 INHIBITOR, CX-4945, SENSITIZES MEDULLOBLASTOMA TO TEMOZOLOMIDE

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Medulloblastoma (MB) is the most common malignant pediatric brain tumor, accounting for ~20% of all cases. While current treatments result in

a cure rate of 70–75%, the surviving patients are afflicted with neurocognitive impairment, endocrine dysfunction, and a severe decrease in quality of life. Consequently, better and more effective treatments are needed to treat these young patients. Casein kinase 2 (CK2) is an intriguing therapeutic target because MB patients with elevated levels of CK2 expression have a significantly worse prognosis. To elucidate the role of CK2 in MB, we modulated CK2 expression in multiple MB cell lines (Daoy and Med1-MB). We discovered that exogenous expression of either CK2 isoform, CK2a or CK2b, increased MB cell growth and significantly decreased survival in mice that were injected intracranially with the modulated cell lines. Consistently, we demonstrated that knocking down CK2 expression using CRISPR-Cas9 or inhibiting CK2 activity using the CK2 inhibitor, CX-4945, decreased MB growth. We conducted a high throughput to identify new compounds that could work synergistically with CX-4945. We screened over 4,000 FDA approved compounds and determined that temozolomide (TMZ) enhanced the efficacy of CX-4945. We corroborated these findings when we verified that knocking out CK2a or CK2b sensitized Daoy cells to TMZ treatment. Interestingly we also determined that combinatorial treatment of CX-4945 and TMZ enhanced MB apoptosis and decreased cell growth. To elucidate the mechanism by which CK2 inhibition sensitized MB cells to TMZ we analyzed the expression of b-catenin, a known regulator of O-6-methylguanine-DNA methyltransferase (MGMT) activity. We determined that inhibition or loss of CK2 activity reduced b-catenin expression, which in turn lead to a decrease in MGMT expression. Together, our findings suggest that CK2 is a novel therapeutic target for MB and that combining CX-4945 and TMZ can lead to a promising new MB therapy.

CSIG-45. RanBPM AS A PARTNER OF STAT5 REGULATES IFN-λ SIGNALING PATHWAY

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BACKGROUND: RanBPM (Ran-binding protein in the microtubule-organizing center, also known as Ranbp9) is a ubiquitous, nucleocytoplasmic protein whose function is poorly understood. RanBPM acts as a multi-modular scaffolding protein, bridging interactions between the cytoplasmic domains of a variety of membrane receptors and their intracellular signaling targets. In glioma rapid increases in the tyrosine phosphorylation of signal transducers and activators of transcription 5 (STAT5) proteins have been extensively documented in cells stimulated with cytokines and growth factors. However, the mechanisms by which STAT5 translocates to the nucleus and regulates proliferation in human glioblastoma multiforme cells have not been studied in detail. In this study, we are just discussing the interaction of RanBPM and STAT5. METHODS: To confirm RanBPM as a binding partner of STAT5, GST pull-down and co-immunoprecipitation (Co-IP) assay were applied. And to map the binding site of the two proteins, various truncated plasmids were constructed and these plasmids were transfected with full length gene of RanBPM or STAT5, respectively. To reveal the biological function of RanBPM after interacting with STAT5A, we employed a luciferase reporter linked with a STAT5 binding element to examine the transcriptional activity of STAT5. RESULTS: In the GST pull-down and Co-IP assay, we confirm RanBPM can interact with STAT5. In the binding site experiment, the result revealed that the two domains of RanBPM, RanBPM-N and RanBPM-C were responsible for the linkage with STAT5. And the conserved binding sites of STAT5 take part in this association. In the dual-luciferase assay, the luciferase activity indicated that the overexpression of RanBPM enhanced the transcriptional activity of STAT5 in glioma cells (U87 MG and U251 MG). From these data, we conclude that RanBPM interact with STAT5 and plays a novel role in regulating transcriptional activity of STAT5.

CSIG-46. DEVELOPMENT OF A NOVEL GLIOBLASTOMA MODEL TO STUDY THE ROLE OF NOX4 IN DISEASE PATHOGENESIS

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Glioblastoma multiforme (GBM), a cancer of the glial cells in the brain, is the most common and aggressive primary brain tumor and even with aggressive and invasive treatment only has a median survival of about a year. The reactive oxygen species producing NADPH Oxidase 4 (NOX4) has been shown to be significantly increased in GBM and patients with high NOX4 expression have shown reduced progression-free survival. However, the molecular mechanism underlying the functional role of NOX4 in this disease is largely unknown. To study this, we have developed a novel *in vivo* model of GBM in WT and GFAP-specific NOX4 KO background by injecting a single *Cre*-inducible lentiviral vector targeting multiple pathways reported in human GBM (classical subtype). This approach allows us to faithfully model the complex contextual signaling events that drive the human pathology, providing a clear picture of relevant pathways and to generate *in vivo* and *in vitro* models that phenocopy the human disease. Our preliminary data indicates that NOX4 blockade prevents glioma stem cell (GSC) differentiation, reduces proliferation, induces cell death and slows disease progression, in part through the PI(3)K/Akt signaling pathway. Elucidation of the NOX4 pathway in GBM will improve our understanding of the cellular signaling mechanisms driving this disease and may offer new therapeutic avenues.

CSIG-47. TRIM24 IS AN ONCOGENIC TRANSCRIPTIONAL CO-ACTIVATOR OF STAT3 IN GLIOBLASTOMA

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Aberrant amplification and mutations of epidermal growth factor receptor (EGFR) are the most common oncogenic events in glioblastoma (GBM), but the mechanisms by which they promote aggressive pathogenesis are not well understood. Here, we determine that non-canonical histone signature acetylated H3 lysine 23 (H3K23ac)-binding protein tripartite motif-containing 24 (TRIM24) is upregulated in clinical GBM specimens and required for EGFR-driven tumorigenesis. In multiple glioma cell lines and patient-derived glioma stem cells (GSCs), EGFR signaling promotes H3K23 acetylation and association with TRIM24. Consequently, TRIM24 functions as a transcriptional co-activator and recruits STAT3, leading to stabilized STAT3-chromatin interactions and subsequent activation of STAT3 downstream signaling, thereby enhancing EGFR-driven tumorigenesis. Additionally, H3K23 acetylation also recruits TRIM24 to activate *PIK3CA* transcription, thereby enhancing PI3K/AKT signaling and tumorigenesis. Moreover, lysine acetyltransferase KAT6A, a chromatin regulator which contributes to histone modification and cancer, is upregulated by EGFR activation and acetylates H3K23 in gliomas. KAT6A expression is associated with GBM patient survival and upregulated by EGFR signaling. KAT6A silencing suppressed cell proliferation, cell migration, colony formation and tumor development in an orthotopic mouse xenograft model system. Taken together, our findings uncover TRIM24 functions as an epigentic oncogene in glioblastoma and suggest TRIM24 as a potential therapeutic target for GBM that are associated with EGFR activation.

CSIG-48. PHOSPHORYLATION OF EZH2 PROMOTES GLIOBLASTOMA STEM-LIKE CELLS SELF-RENEWAL THROUGH NF-κB METHYLATION

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PURPOSE: The oncogenic role of MELK/EZH2/NF-κB signaling in the self-proliferation of glioma stem-like cells (GSCs) was discovered to validate the pathway as a therapeutic target and prognostic candidate for glioblastoma. EXPERIMENTAL DESIGN: Expressing exploration and survival analysis were performed to evaluate the association of MELK/EZH2/NF-κB enrichment with the prognostic value in glioma samples. The dynamic expression of MELK/EZH2/NF-κB at the stem-cell assembled regions during mouse brain development was detected to estimate the proliferative potential of the axis. Mechanistic interacting course was examined using the RNA knockdown and immunoblot techniques to confirm the signaling impacting the GSCs self-proliferation. The progression of xenografts derived from human GSCs was examined after genetic or drug blockage of MELK/EZH2/NF-κB signaling in GSCs. RESULTS: We reported that EZH2 phosphorylated by MELK bound to NF-κB methylating it in GSCs, which promoted its ability to mediate the development of glioblastoma. Striking parallels regarding the enrichment of MELK/EZH2/NF-κB axis between GSCs and normal stem cells indicated the association of the pathway with stemness. Clinically, the proportion of MEKL/EZH2/NF-κB signaling elevated progressively during the increasing glioma grades and could be considered as the potential indicator of survival. In addition, functional loss of this axis effectively impaired the proliferation of GSCs. CONCLUSION: Our findings show that the MELK/EZH2/NF-κB interaction is required for the GSCs derived neoplastic proliferation, thereby uncovering in the signaling a therapeutic candidate to treat this malignancy.

CNS METASTASIS

CMET-01. CLINICAL AND DOSIMETRIC FACTORS RELATED TO RADIATION NECROSIS AFTER FIVE FRACTION RADIOSURGERY FOR RESECTED BRAIN METASTASES

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PURPOSE: Stereotactic Radiosurgery (SRS) is increasingly utilized in the management of resected brain metastases (rBM). A significant complication is radiation necrosis (RN) due to larger target size. Despite dose de-escalation and hypofractionation, rates of RN after SRS to rBM remain high. The aim of this analysis is to evaluate dosimetric parameters associated with RN for rBM. Methods: From 2008–2016, 55 rBM in 52 patients at a single institution that were treated with 5 fraction LINAC based SRS (25-35Gy) with a minimum 3 months follow-up were evaluated. For each lesion, variables including clinical target volume (CTV), dose and location/magnitude of hot spots were recorded. Hot spot location was stratified as either within tumor bed (CTV) or within the PTV expansion margin (PTV minus CTV). Overall survival (OS) estimated using Kaplan-Meier method. Cumulative incidence with competing risks was used to estimate rates of RN and local recurrence (LR). Optimal cut-points predicting for RN for hotspot magnitude based on location were identified via maximization of the log-rank test statistic. Results: Median age and OS for all patients was 58.5 years and 16.2 months, respectively. For all targets, the median CTV was 17.53 cc, and mean max hotspot was 113%. At 1 year, cumulative incidence of RN and LR for all patients was 21.8% and 13.1%. Univariate analysis showed max hot spot (hazard ratio (HR): 3.28, p=0.045) and hot spots within PTV expansion margin of 105%, 110% and 111% predicted for RN with HRs of 3.64, 8.47, and 6.90 respectively (all p<0.05), but hot spots within the CTV did not. Conclusion: To our knowledge, this is the first study that investigated dosimetric factors that predict for RN after hypofractionated SRS to rBM. Hot spot location and magnitude appear important for predicting RN risk, suggesting these parameters should be considered during treatment planning.

CMET-03. PHASE 1 TRIAL OF OSIMERTINIB WITH STEREOTACTIC RADIOSURGERY IN EGFR MUTANT LUNG CANCER BRAIN METASTASIS

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BACKGROUND: Brain metastases (BM) occurs in 25–40% of patients with non-small cell lung cancer (NSCLC). Traditionally patients with brain metastases were treated with whole brain radiation therapy (WBRT), stereotactic radiosurgery (SRS) and surgical resection. Historically, BM is associated with dismal prognosis. Recent advances in the targeted therapy have improved BM outcomes. In patients with epidermal growth factor receptor (EGFR) positive NSCLC with 1–10 BM, we hypothesize to control the macro BM with SRS and osimertinib. Osimertinib use will help control the micro metastases. This approach would avoid WBRT that can potentially lead to significant congnitive decline. METHODS: This is single arm, phase I multicenter study of Osimertinib with SRS. Primary endpoint is to determine the safety of Osimertinib in combination with SRS EGFR positive NSCLC with BM. Secondary end points include 6-month intracranial and extracranial progression free survival (PFS-6), overall survival (OS) in EGFR positive NSCLC BM. To compare results of this clinical trial to historical controls of EGFR positive NSCLC BM treated with SRS alone. To assess the intracranial and extracranial overall response rate as measured by RECIST 1.1. Eligibility criteria includes age >18 years, historically confirmed EGFR mutation positive NSCLC with newly diagnosed BM, 1–10 BM, ECOG status of 0–2. Key exclusion criteria include leptomeningeal disease, BM within 5 mm of optic chiasma or optic nerve, metastases in brain stem, history significant cardiovascular and other comorbidities, known HIV or chronic hepatitis infection and those receiving other investigational cancer therapy. Statistical analysis: The study will employ a 3 + 3 design with an expansion cohort at the maximum tolerated dose (MTD) and up-to 40 patients will be enrolled to determine safety and preliminary efficacy with the combination. RESULTS: The study (CASE 3517) is ongoing and enrolling patient with EGFR mutant NSCLC.

CMET-04. METASTATIC GASTROINTESTINAL CANCER TO THE SKULL BASE MIMICKING NF2

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Cancers of gastrointestinal (GI) origin rarely metastasize to the brain and is present in less than 1% of patients at diagnosis. We present a case of GI cancer presenting with bilateral cerebellopontine angle masses resembling bilateral vestibular schwannomas (VS) seen in Neurofibromatosis type 2 (NF2). A 40 year-old woman presented to our NF2 multidisciplinary clinic after 4 months of vertigo, unilateral hearing loss and facial paralysis. Brain MRI was consistent with bilateral vestibular schwannomas with no other findings. Her symptoms then progressed over 2 months with bilateral hearing loss and facial paralysis despite two treatments with bevacizumab, and repeated imaging revealed tumor enlargement bilaterally. The patient underwent a resection of the larger, right-sided skull base mass, and pathology revealed an adenocarcinoma of gastrointestinal origin. FDG-PET and CT of the chest, abdomen, and pelvis revealed diffused lymphadenopathy in the abdomen. The patient also had an elevated CA 19-9 level. Next generation sequencing of the resected skull base mass was most consistent with a tumor of GI origin. This is the first known case of metastatic GI cancer to the skull base resembling the bilateral VS seen in NF2.

CMET-05. ASSOCIATION BETWEEN TUMOR LOCATION AND TOXICITY OUTCOMES AFTER STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES

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OBJECTIVE: The toxicities associated with stereotactic radiosurgery (SRS) are important factors when considering treatment options and supportive management for patients with brain metastases. We assessed the association between brain metastasis location and rates of toxicity after SRS. METHODS: We conducted a retrospective single-institution review of 170 patients treated with SRS for brain metastases from 2008–2016 with median follow-up of 8.6 months. Typical SRS doses were 18-20Gy in 1 fraction (lesions <2cm), 18-21Gy in 3 fractions (lesions 2-3cm), and 25-30Gy in 5 fractions (lesions > 3cm). Toxicity measures evaluated included radiation necrosis, seizure, and dexamethasone requirement. RESULTS: A total of 221 lesions were treated among frontal (29%), cerebellar (23%), parietal (16%), temporal (15%), occipital (14%), and other (brainstem, thalamus, basal ganglia) (4%) regions. The rate of SRS-related radionecrosis was 4% for all patients and significantly correlated with metastasis volume (increasing from 1% to 7% for lesions ≤1cm^3 to > 3cm^3) and prior whole brain radiotherapy (WBRT) but not with metastasis location or prior resection on multi-variable analysis (P<0.05). Post-SRS seizure occurred in 9% of all patients but was significantly higher after SRS to primary motor cortex and sensory cortex lesions, associated with 50% and 33% seizure rates, respectively (P<0.05). Of patients who initially presented with seizure and were on anti-epileptic medication during SRS, 62% had no further seizures, while 38% did have post-SRS seizures, nearly all with motor cortex lesions. Only 5% of patients had new-onset seizure after SRS, related to lesion hemorrhage or motor cortex location. Dexamethasone use > 3 months post-SRS was higher for motor strip lesions. CONCLUSION: Brain metastasis location in the primary motor cortex was associated with higher rates of post-SRS seizure, including new-onset seizures and breakthrough seizures on anti-epileptic medication during SRS. Rates of radionecrosis were associated with lesion volume and prior WBRT but not with metastasis location.

CMET-06. DISTANT BRAIN FAILURE AND SALVAGE FREE SURVIVAL FOR RADIOSURGERY-TREATED MELANOMA BRAIN METASTASES IN THE ERA OF CHECKPOINT INHIBITOR IMMUNOTHERAPY

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PURPOSE: We evaluated median time-to-distant brain failure (DBF), and salvage-free survival from distant brain failure (DBF-SFS) in melanoma brain metastases (MBM) patients who received stereotactic radiosurgery (SRS) and checkpoint inhibitor treatment. METHODS: An IRB-approved retrospective evaluation of 68 MBM patients with 229 metastases treated with SRS between 11/2008 and 2/2017 with at least one post-SRS brain MRI. Time-to-DBF was the interval between initial SRS to MRI revealing

any new brain metastases. DBF-SFS was defined as the interval from initial SRS to MRI revealing brain metastases requiring salvage treatment. Survival analysis was performed using Kaplan-Meier estimates and Cox regression. RESULTS: Overall median time-to-DBF was 4.34 months. Median time-to-DBF for patients who received PD-1 inhibitors (5.43mo), ipilimumab without PD-1 inhibitors (3.95mo), and no immunotherapy (3.30mo) were not significantly different (p=0.28). Median overall survival of non-immunotherapy patients was 3.29 months and all DBF occurred within 4 months in this subgroup. Patients with active extracranial primary disease had significantly worse median time-to-DBF (3.58mo) than those without active primary disease (9.90mo) (HR 3.25, p< 0.01). Overall median DBF-SFS was 4.50 months. Median DBF-SFS for patients who received PD-1 inhibitors (9.21mo), ipilimumab without PD-1 inhibitors (5.44mo), and no immunotherapy (4.50mo) were not significantly different. Multivariate analysis confirmed a significantly worse DBF-SFS for patients with > 2 SRS-treated metastases (2.30mo) versus 1–2 metastases (9.21mo), (HR 10.22, p< 0.01). Patients treated in 2014 or later demonstrated significantly longer DBF-SFS (median not reached) vs those treated prior to 2014 (4.34mo) (HR 0.01,p=0.01). CONCLUSIONS: Our study demonstrated improving DBF-SFS rates for patients treated since 2014 compared to those treated earlier. Our study does find that patients with active extracranial disease and > 2 initial metastases have higher rates of DBF and DBF-SFS.

CMET-07. FRAILTY PREDICTS MORTALITY AFTER RESECTION OF BRAIN METASTASES

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INTRODUCTION: Brain metastases are the most common type of brain tumor, though determining candidates for resection may be challenging. Patients' unfavorable prognoses make pre-operative risk stratification critical in the selection of patients that are likely to benefit from resection. METHODS: Multivariable logistic regression was applied to 3,532 cases of secondary neoplasms of the brain in the American College of Surgeons National Surgical Quality Improvement Program database. The validated 5-criteria modified frailty index (mFI-5) score was utilized to quantify frailty, characterized as an mFI-5 score of 2 or higher. RESULTS: The median age in the cohort was 61 years, and the majority of patients were female (55%). Frailty was present in 17% of patients. The most frequent 30-day post-operative medical complications were venous thromboembolism (3.1%), pneumonia (2.7%), and urinary tract infections (2.2%). Unplanned readmissions and reoperations occurred in 12.2% of patients and 4.9% of patients, respectively. The incidence of death was 4.2% across the cohort, including 1.2% during index hospitalization. Frailty was associated with pneumonia (OR 2.7, p DISCUSSION: Frailty was associated with post-operative medical complications and death, in particular during the post-discharge phase. Frailty should be used in risk-stratifying patients.

CMET-08. BRAIN METASTASES AS PRIMARY PRESENTATION OF SOLID TUMORS: A DESCRIPTIVE ANALYSIS OF THE NATIONAL CANCER DATABASE (NCDB)

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INTRODUCTION: Survival of cancer patients with brain metastases (BM) has increased with novel therapies. Previous analyses have focus on radiation treatment or predate novel systemic therapies that have improved survival outcomes. METHODS: We analyzed the National Cancer Database from 2010–2014, for all patients with metastatic disease to the brain to analyze patterns of care across the United States. RESULTS: 88,459 cases were included. The majority of patients were male (51.7%), white (84.2%), and non-Hispanic (92.6%), with a median age of 65 years. The most common primary cancer sites included lung and upper airway cancer (82.5%), breast cancer (4.2%), skin cancer (3.5%), and gastrointestinal tumors (2.3%). Hepatobiliary cancer patients had a higher frequency of comorbidities (9.6% with a Charlson-Deyo score ≥3). Patients were more likely to be treated in community centers (64.0%) and lived in metropolitan areas (79.0%). Overall 72.6% received any form of radiation, 53.5% received radiation to the brain, 52.1% received chemotherapy, 2.3% received immunotherapy, and 13.3% underwent surgical procedure to distant metastatic site. The most common radiation modality was whole-brain radiation (58.4%), followed by stereotactic radiosurgery (11.6%), other (0.65), and brachytherapy (0.1%). The type of radiation was unknown in 2.0% of the patients. Radiation to the brain was more common in skin cancer (63.4%). The use of chemotherapy was significantly lower in patients with hepatobiliary cancer (34.2%). Immunotherapy was used in 2.3% of patients, mainly in melanoma (13.6%), and breast cancer (8.15%). Median survival for all cases was 5 months, and was significantly higher in male reproductive system cancer (16.3 months), and lower in hepatobiliary cancer (2.7 months). CONCLUSIONS: The increased survival of BM in the current era exceeds historical RTOG recursive partitioning analysis. Large scale data, such as NCDB, is essential for single tumor analysis with treatment and survival data, and provides a reference for future investigations.

CMET-09. IMPACT OF STEROIDS ON THE EFFICACY OF IMMUNE CHECKPOINT INHIBITORS IN PATIENTS WITH NON-SMALL CELL LUNG CANCER BRAIN METASTASES

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BACKGROUND: Non-small cell lung cancer brain metastases (NSCLCBM) have dismal outcome. Immune checkpoint inhibitor (ICI) has promising activity in lung cancer; however, limited data exists on impact of steroids on efficacy of ICI in NSCLCBM. METHOD: We reviewed 120 NSCLCBM patients treated with ICI (2012–2017) at our center. Fifty-nine patients who received at-least 2 cycles of ICI after diagnosis of NSCLCBM with at least one follow-up MRI were included for analysis, others were excluded as they have received ICI before BM or did not have follow-up MRI. All patients had prior chemotherapy and received ICI (nivolumab/pembrolizumab) in second or third line. Overall survival (OS) was calculated from date of ICI therapy to date of death or last follow-up. Progression free survival (PFS) was calculated from date of ICI to date of progression. OS was estimated by Kaplan-Meier method and analyzed by Cox proportional hazards model. RESULTS: Median age was 61 years (39–77 years), median KPS of 90, 59% were females, 49 were adenocarcinoma (4 EGFR mutations, 2 ALK rearrangement). Forty-one patients had supratentorial lesions, 7 had infratentorial and 11 had both. Fifty-four patients underwent stereotactic-radiosurgery, 4 had whole brain radiation-therapy and 30 patients received steroids (28 at baseline before start of ICI and 2 patients within 2 cycles of ICI). Twenty-two patients received dexamethasone (2–8 mg/day), 8 received prednisone (5–20 mg/day). Median OS was 18.9 months and PFS was 9.9 months. Steroid use was associated with decreased PFS, 5.0 months vs 13.8 months (p value = 0.02). No significant difference was seen in OS with steroid treatment. On multivariable analysis, age at diagnosis significantly predicted OS ($p = .034$). Greater number of baseline intracranial lesions ($p < .01$) and concurrent steroids ($p < .0005$) correlated with decreased PFS. CONCLUSION: Steroid use in NSCLCBM is associated with decreased PFS with ICI.

CMET-10. RISK OF RADIATION NECROSIS FOLLOWING REPEAT STEREOTACTIC RADIOSURGERY FOR RECURRENT BRAIN METASTASES

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INTRODUCTION: Stereotactic radiosurgery (SRS) has become the standard of care for the treatment of many patients with oligo-metastases of the brain with a local control rate of 80–90% and radiation necrosis rate of 7% per lesion. Per patient, the rate of radiation necrosis is 10–15% with half being symptomatic. We sought to determine outcomes of stereotactic radiosurgery for treatment of recurrent brain metastases after failure of initial SRS. METHODS: Retrospective review of all patients that underwent Gamma Knife radiosurgery at our institution between 2000 and 2018 was conducted. We screened 2,671 patients and identified 51 lesions in 39 patients that recurred after the first treatment and received a second single-fraction stereotactic radiosurgery dose to the same lesion. RESULTS: The median patient follow-up after the second radiosurgery treatment was 10.2 months (1.1–60.5 months). The median time between the two treatments was 16.8 months (range: 2.5–75.3 months). The diagnosis of progression was based on imaging changes on PET and CBV or biopsy. The average radiation dose at first and second treatments was 21 Gy and 19 Gy, respectively. Out of 51 lesions that received two SRS treatments, 25 (49%) increased in size within a median of 4.8 months post second treatment (range: 1.0–29.4 months). Of these, 18 lesions (35%) in 17 patients (43%) were diagnosed as radiation necrosis based on either a pathological examination (7 lesions), or a combination of radiographic findings (11 lesions). The rates of symptomatic radiation necrosis were 16% per lesion and 21% per patient. CONCLUSION: Repeat SRS treatment of recurrent brain metastases is associated with a higher rate of radiation necrosis. Increase in size post repeat SRS is more likely radiation necrosis than recurrence. Strategies such as dose reduction, fractionated treatments, or combination with laser ablation may improve outcomes and reduce the rate of subsequent radiation necrosis.

CMET-11. LEPTOMENINGEAL MELANOMA METASTASIS PRESENTING AS INTRAVENTRICULAR AND SUBARACHNOID HEMORRHAGE

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Leptomeningeal spread of cancer can have protean presentations, increasing the difficulty of making this diagnosis. We present the case of a 77-year-old man with prior metastatic melanoma who presented with spontaneous headache that worsened over several days, and was found to have both intraventricular and cortical subarachnoid hemorrhage on CT head. His neurological exam was unremarkable. MRI of the brain with contrast confirmed the CT findings and did not reveal any evidence of parenchymal or leptomeningeal metastases. MR angiogram followed by a conventional angiogram did not demonstrate any vascular abnormalities. Cerebrospinal fluid examination revealed 110 white blood cells, 34000 red blood cells, and protein of 160. Cytology returned positive for malignant melanoma cells. No additional sites of disease were found on further evaluation with MRI spine and CT of the chest, abdomen and pelvis. In retrospect, the patient had endorsed some fluctuating nausea and fatigue three months prior to presentation, as well as positional vertigo two weeks prior to presentation. His oncologic history was notable for prior immunotherapy with ipilimumab, as well as diagnosis of dural-based brain metastases two years earlier, for which he underwent surgical resection and radiation therapy. He had no evidence of disease for one year prior to this new diagnosis of leptomeningeal involvement. This case highlights an unusual presentation of leptomeningeal metastasis and underscores the importance of maintaining heightened suspicion for this diagnosis when evaluating subarachnoid hemorrhage in a cancer patient, especially in the case of melanoma. It raises the question of whether this particular cancer type or the patient's prior immunotherapy contributed to his presentation, and emphasizes the need for further elucidation of the biology of leptomeningeal metastasis.

CMET-12. DISTANT BRAIN FAILURE FOLLOWING STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES FROM BREAST CANCER

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PURPOSE: We examined distant brain failure (DBF) outcomes among patients treated with SRS for breast cancer brain metastases. METHODS: We completed a retrospective IRB-approved review of 75 breast cancer patients treated with SRS for 271 brain metastases. Median time to DBF was defined as time from initial SRS to MRI revealing a new lesion. Kaplan-Meier and Cox proportional hazards model were used for statistical evaluation. Of the 75 patients, 74 patients were classified with ER status (46 positive vs 28 negative), PR status (31 positive vs. 43 negative), and HER2-Neu status (35 positive vs. 39 negative). 43 patients (57%) had uncontrolled extracranial disease at time of initial SRS. 18 patients (24%) received prior WBRT and 5 patients (7%) received concurrent WBRT. RESULTS: The median time to development of 1 brain metastasis was 7 months, with 7 patients (9%) developing only 1 additional brain metastasis and 40 patients (53%) developing greater than 1 additional brain metastasis. Median time to development of 2–4 brain metastases was 15 months, with 10 patients (13%) developing only 2–4 additional brain metastases and 30 patients (40%) developing greater than 4 additional brain metastases. Median time to development of greater than 4 brain metastases was 22 months. Median time to leptomeningeal disease was not reached, with 19 patients (25%) developing LMD. PR positivity predicted for greater time to development of 1 new brain metastasis (p=0.025). Extracranial disease control was a positive prognostic factor for time to development of 1 new brain metastasis (p=0.046), 2–4 new brain metastases (p=0.004), and greater than 4 new brain metastases (p=0.004). CONCLUSION: Our results suggest nearly 2/3 of breast cancer patients treated with SRS for brain metastases will develop at least one additional metastasis. Prognostic factors for DBF for these patients include PR status and extracranial disease control.

CMET-14. DISTANT BRAIN FAILURE FOLLOWING STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES FROM NON-SMALL CELL LUNG CANCER

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PURPOSE: We examined distant brain failure rates and underlying prognostic factors among patients treated with SRS for brain metastases from NSCLC. METHODS: We performed an IRB-approved retrospective study of 178 NSCLC patients treated with Linear Accelerator-based stereotactic radiosurgery (SRS) for 526 brain metastases. Median time to distant brain failure (DBF) was defined as time from initial SRS to MRI revealing a new lesion. Kaplan Meier and Cox proportional hazards model were used for statistical evaluation. All but 10 patients were classified by histology, with the majority (76%) classified as adenocarcinoma. 116 patients (65%) had uncontrolled extracranial disease. 22 patients (12%) received previous WBRT and 15 patients (8%) received concurrent WBRT. 6 patients were ALK positive and 63 were ALK-negative. 26 patients were EGFR positive and 64 were EGFR negative. RESULTS: The median time to development of 1 brain metastasis was 8 months, with 22 patients (12%) only developing 1 additional brain metastasis and 89 patients (50%) developing greater than 1 additional brain metastasis. Median time to development of 2–4 brain metastases was 12 months, with 39 patients (22%) developing only 2–4 additional brain metastases and 50 patients (28%) developing greater than 4 additional brain metastases. Median time to development of greater than 4 additional brain metastases was 26 months. Median time to leptomeningeal disease (LMD) was 62 months, with 21 patients (12%) developing LMD. On univariate analysis, adenocarcinoma histology (p=0.031), EGFR mutation negative or unknown (p=0.042), and controlled extracranial disease (p=0.011) were positive prognostic factors for time to development of 1 additional brain metastasis. CONCLUSION: We report the incidence of distant brain failure among lung cancer patients treated with SRS. Our study revealed that the probability of distant brain failure is increased for those with EGFR mutation, active extracranial disease, or non-adenocarcinoma histology.

CMET-15. WHOLE EXOME SEQUENCING OF BRAIN METASTASES FROM COLORECTAL PRIMARY CANCERS REVEALS CLINICALLY ACTIONABLE MUTATIONS

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BACKGROUND: Current understanding of the underlying genetic evolution in metastatic colorectal cancer to the central nervous system is lacking. Further, there are no specific treatments for brain metastasis derived from primary gastrointestinal malignancies. Here, we report preliminary data using a next generation sequencing approach to characterize actionable genomic targets in matched colorectal primaries and their associated extra- and intracranial metastases. METHODS: A growing cohort of metastatic colorectal cancer is being assembled that consists of a primary tumor, at least a single extracranial metastasis, an intracranial metastasis, and a normal tissue control for each patient. Nucleic acid was extracted for use in whole exome sequencing from twelve samples. Somatic variants in the primary tissue were identified relative to the matched control sample and further compared relative to the metastatic samples. Initial analysis has focused on cancer genes that have been established to have clinical implications to identify variants with potential therapeutic value. RESULTS: Complex variant signatures were found across the primary colorectal tumors. At least one clinically actionable variant was identified in each case. Alterations in *AKT1, POLE, BRAF* and *GNAS* were detected in the brain metastasis samples and not in the extracranial sites. Alterations in *GNAS, ARID1A, RET,* and *FGFR2* occurred at a higher frequency in metastatic samples (extracranial and brain metastases) compared to primary. *KRAS* and *PIK3CA* status was concordant across all tumors while *PTEN* and *BRAF* status was variable. CONCLUSIONS: Clinically actionable mutations can be found in brain and extracranial metastases that are not detected in their respective clinically sampled colorectal primary tumor. This provides support for the development of combined targeted therapeutic strategies that may be more successful in the metastatic setting. Further investigation is required in larger cohorts to fully characterize the genetic landscape and potential drivers of brain metastasis from colorectal cancer.

CMET-16. THE ROLE OF SURGICAL RESECTION OF MELANOMA BRAIN METASTASES IN THE IMMUNOTHERAPY ERA

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Immune checkpoint blockade has systemic efficacy in patients with metastatic melanoma, including those with brain metastases. However, immunotherapy-induced intracranial tumoral inflammation can lead to neurologic compromise, requiring steroids, which abrogate the systemic efficacy of this approach. We hypothesize that early surgical intervention creates an opportunity for improved survival amongst patients undergoing immune checkpoint blockade for metastatic melanoma. An IRB approved, single institution retrospective study identified 142 patients with melanoma brain metastases treated with immune checkpoint blockade. Overall survival was calculated from date of diagnosis of brain metastasis until death from any cause. Model building included a prognostic model of overall survival and the effect of sequencing of immunotherapy and surgery on overall survival. The 2-year overall survival for patients treated with CTLA-4, PD-1 or combinatorial blockade were 19%, 54%, and 57%, respectively. Patients undergoing surgery for melanoma brain metastases prior to immunotherapy had a median survival of 22.7 months (95% CI: 12.6 to 39.2) compared to 9.3 months (95% CI: 5.7 to 31.1) for patients undergoing surgery after immunotherapy (P=0.06). Amongst surgical patients, the sequence of immunotherapy, diagnosis of brain metastases, and surgery was significantly associated with the hazard of death (P=0.002). Surgery for treatment-naïve intracranial disease followed by immunotherapy is associated with increased overall survival compared to patients who underwent surgery for brain metastases that developed on immunotherapy (HR: 2.96, 95% CI: 1.4 to 6.1). These results suggest that in treatment-naïve patients, early surgical resection for local control should be considered prior to commencing immunotherapy.

CMET-17. VENTRICULOMEGALY AFTER STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES

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Radiation therapy is a known risk factor for leukoencephalopathy and ventriculomegaly in patients with brain metastasis (BM). However, whether repeat stereotactic radiosurgery (SRS) contributes to these complications remains poorly studied. The question is pertinent since the cerebrum is exposed to a variable amount of radiation during each round of SRS. We performed a retrospective analysis of patients who underwent SRS for BM between 2007 and 2017 at our institution and had > 3 months of MRI follow-up. MRIs were assessed for ventriculomegaly based on published morphometric criteria. Statistical analysis was accomplished using Student's t-test, Pearson's chi-squared test, and univariate and multivariate logistic regression. We identified 214 patients who underwent 1,106 SRS for BM. Sixty-three patients (29%) presented with ventriculomegaly prior to SRS. Risk factors for presenting with ventriculomegaly prior to first SRS were female sex (odds ratio (OR) 0.373, 95% CI 0.176 - 0.767, p=0.008) and older age (OR 1.096, 95% CI 1.06 - 1.137, p< 0.001). Of the other 151 patients with normal ventricular size at the time of SRS, 29 patients (19%) developed ventriculomegaly after SRS. Of the 29 patients who developed ventriculomegaly, ten patients (34%) required operative CSF diversion for symptomatic relief. We identified two risk factors that were associated with increased odds of ventriculomegaly: receiving > 4 rounds of SRS (OR 4.1, 95% CI 0.8 - 20.6, p=0.038) and prior history of whole-brain radiation therapy (WBRT, OR 5.6, 95% CI 2.3 – 13.9, p< 0.001). The association between > 4 rounds of SRS and ventriculomegaly remained robust in patients without prior history of WBRT, which suggests that both forms of radiation contribute to the risk of developing new ventriculomegaly. Our results suggest that prior history of WBRT and > 4 rounds of SRS independently contribute to the risk of ventriculomegaly in SRS-treated BM patients.

CMET-18. RISK FACTORS FOR CEREBRAL EDEMA AFTER STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES

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Adverse radiation effect (ARE) after stereotactic radiosurgery (SRS) for brain metastasis (BM) patients is a dreaded complication that often leads to significant compromise in the patient's quality of life. Here, we sought to determine risk factors associated with ARE following SRS. We identified 214 BM patients (1,106 BM) treated between 2007 and 2017 at our institution with > 3 months of MRI follow up. We collected pertinent demographic, clinical, and SRS parameters. ARE was defined as the presence of FLAIR hyperintensity in > 25% of the area of any of four axial MRI images defined by 1) the centrum semiovale, 2) third ventricle, 3) temporal horns, and 4) the fourth ventricle. Statistical analysis was carried out using Student's *t*-test, Pearson's chi-squared test, and univariate and multivariate logistic regression. Of the 214 patients, 62 patients (28.97%) suffered ARE following SRS. On univariate logistic regression, the odds of ARE were increased with higher cumulative intracranial tumor volume (CITV) (odds ratio (OR) 1.026 per 1 cm^3 increase, 95% CI 1.009 - 1.043, p= 0.003), > 4 rounds of SRS sessions (OR 9.545, 95% CI 1.924 - 47.352, p=0.006), and prior history of whole-brain radiation therapy (WBRT) (OR 4.459, 95% CI 2.233 - 8.907, p< 0.001). On multivariate logistic regression adjusted for length of follow-up, these associations remain robust: CITV (p=0.036), > 4 rounds of SRS (p=0.033), and prior history of WBRT (p< 0.001). Variance analysis indicated that the relative importance of these risk factors in contributing to ARE, in descending order, is: prior history of WBRT, CITV, and > 4 rounds of SRS. In this analysis of our decade-long experience, we discovered three risk factors for ARE, including prior history of WBRT, increasing CITV, and > 4 rounds of SRS. Consideration of these factors should facilitate patient counseling and clinical decision making.

CMET-19. CLINICAL RISK ASSESSMENT SCORE TO ESTIMATE THE LIKELIHOOD OF PSEUDOPROGRESSION VERSUS TUMOR GROWTH FOLLOWING STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASES

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A major challenge in the follow-up of patients managed with stereotactic radiosurgery (SRS) brain for metastases (BM) is to differentiate pseudoprogression (PP) from tumor recurrence (TR). A clinical score based on tumor and treatment related factors would be valuable when selecting appropriate treatment. Follow-up images of 97 consecutive patients treated with SRS for 406 BM were analyzed. We included 100 (24.6 %) BM in 42 (43.3 %) patients which responded either with TR (delayed growth; 53 (13.1 %) BM in 27 patients) or PP (temporary volume increase; 47 (11.6 %) BM in 15 patients). Differences between the 2 groups were analyzed and used to develop a PP risk assessment score (PP-RAS). Significant factors associated with a higher incidence of PP versus TR were: primary lung cancer vs. other primaries, BM volume < 2cc (or BM < 1.5 cm in diameter), Target cover ratio ≥ 98 % and prior radiation with SRS or WBRT. Based on the presence (0) or not (1) of these 5 parameters, a risk assessment score for PP versus TR was established. A PP-RAS score of 0 corresponds with high risk of PP vs.TR, whereas a score of 5 corresponds low risk of PP vs. TR. A score of ≤ 1 point was associated with 100 % PP, 2 points with 57 % PP and 43 % TR, 3 points with 57 % TR and 43 % PP whereas ≥ 4 points were associated with 84 % TR and 16 % PP, π=24.57, df =4, p < 0.001). Based on these 5 parameters at the time of SRS our risk assessment score could robustly differentiate between PP versus growth following SRS. The score is user-friendly and may be a useful tool to guide the decision making whether to retreat or observe at appropriate follow-up intervals.

CMET-20. EVIDENCE OF CNS RESPONSE OF PEMBROLIZUMAB FOR LEPTOMENINGEAL CARCINOMATOSIS AT A SINGLE CELL RESOLUTION

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Approximately 8% of patients with cancer develop leptomeningeal carcinomatosis (LMD). LMD is associated with approximately 4 week median survival and a paucity of treatment options beyond palliative shunting. We performed a phase II study of the PD-1 inhibitor pembrolizumab in LMD from any solid tumor malignancy (NCT02886585). The primary endpoint is the rate of overall survival at 3 months (OS3). A Simon two-stage design was used to compare a null hypothesis OS3 of 18% against an alternative of 43%. Serial CSF, blood samples and tumor samples were collected to elucidate the genomic and transcriptional determinants of response to

immunotherapy in central nervous system lesions. A total of 18 patients were accrued and the median follow-up of patients still alive was 6.8 months (range: 2.2 to 7.6 months). At the time of data retrieval, 11 patients (61%) were alive at three months after enrollment (OS3). Therefore, the study met its primary endpoint. Whole exome sequencing of tissue samples and cell-free DNA from CSF and blood, as well as single-cell RNA sequencing of CSF, were carried out to decipher tumor evolution, track immune cell recruitment, and identify biomarkers of response. Analysis of 7877 tumor and immune cells across 6 patients demonstrated patient-specific tumor clustering and evidence of T cell and antigen presenting cells recruited to the CSF following pembrolizumab treatment. Longitudinal CSF samples demonstrated genetic and transcriptomic differences in tumor and immune cells suggesting response to treatment in patients that reached OS3 compared with those that did not. These findings suggest that pembrolizumab has activity in LMD and that CSF provides an opportunity to monitor the clonal evolution of the tumor and immune microenvironment in LMD.

CMET-21. THE ROLE OF BRAIN METASTASIS FREE INTERVAL IN PATIENTS WITH BRAIN METASTASES OF BREAST CARCINOMA

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BACKGROUND: In patients with brain metastases of breast cancer (BMBC), time interval between primary tumor diagnosis and appearance of first brain metastasis varies widely. This brain metastasis free interval (BMFI) is a readily available parameter and has been suggested by some to be of prognostic value. The aim of this study is to compare characteristics between patients with different BMFIs and determine if BMFI is a prognostic factor for overall survival in BMBC. METHODS: We retrospectively reviewed a multi-institutional neurosurgical/radiation oncological database of female patients who were treated for BMBC between 1996 and 2017. Cox proportional hazards model and Kaplan-Meier survival curves were used to determine prognostic value for survival. RESULTS: A total of 503 patients were included. Median age at first brain metastasis was 52 (interquartile range (IQR) 45–58). Median BMFI was 38 months (IQR: 18 - 66) and median overall survival was 17 months (IQR 8 - 31). In univariate cox proportional hazards model, age at brain metastasis, tumor subtype, and the presence of liver or lung metastases were significantly associated with overall survival. BMFI > 3 years was not associated with a longer overall survival (hazard ratio (HR) = 1.13, p = .21). In multivariate analysis, only subtype (luminal-her2 versus triple negative, HR = .77, p = .02) and the presence of liver metastases (HR = 1.36, p = .01) were prognostic for overall survival. Again, no significant association with BMFI was found (HR = .99, p = .91). CONCLUSIONS: In this large, retrospective cohort, patients with a long BMFI had a similar overall survival when compared to those with a shorter interval.

CMET-22. INTRATHECAL (IT) TRAZTUZUMAB (T) FOR THE TREATMENT OF LEPTOMENINGEAL METASTASES (LM) IN PATIENTS (PTS) WITH HUMAN EPIDERMAL GROWTH FACTOR RECEPTOR 2-POSITIVE (HER2+) CANCER: A MULTICENTER PHASE 1/2 STUDY

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Pts with HER2+ breast cancer have frequent LM. No current FDA approvals exist. A multicenter phase I/II trial assessing safety and efficacy of IT T in LM pts was conducted. The primary endpoint in phase 2 was response rate (RR). Complete response (CR) required cytologic CR (CCR) + radiographic CR (RCR) + stable clinical function. Partial response (PR) required either CCR with stable/improved imaging and clinical function or RCR + stable cytology + stable/improving clinical functioning. Pts received IT T via an intraventricular Ommaya reservoir. Phase I dosing started at 10 mg, then increasing by 20 mg up to 80mg. Each cycle (C) was 4 weeks with 2x/week treatment in C1, weekly in C2, and every two weeks after C2. Pts were allowed to continue on hormonal agents if systemic disease was controlled at LM development. Concurrent radiation therapy was not allowed, with the exception of localized treatment for pain control. 34 pts were enrolled with 26 in phase 2. The median age was 51 (25–69). IT T was well tolerated with no DLTs seen throughout; determined MTD was 80 mg for phase 2. All pts treated in phase 2 had Her2+ breast cancer, 2 pts in the phase 1 had non-breast histologies. Median cycles completed was 2 (1–22). Median follow up was 9.1 months (0.4–28.9). In phase 2, 5 pts (19.2%) had PR, 13 (50%) had stable disease (SD) and 8 (30.8%) had progressive disease. For phase 2 pts, median PFS pts was 2.4 months (CI 1.0–5.5) and median OS was 12.1 months (CI 4.3–19.6). IT T was tolerated up to a dose of 80mg. Primary endpoint (25% RR) was not met, however 69% of pts showed clinical benefit (SD or better); median OS exceeded historical controls. Future studies are warranted to evaluate IT T in HER2+ LM.

CMET-23. NATURAL HISTORY AND RISK FACTORS FOR CYSTIC FORMATION AFTER STEREOTACTIC RADIOSURGERY FOR BRAIN METASTASIS

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A comprehensive review of the literature on brain metastasis (BM) stereotactic radiosurgery (SRS) revealed no publications on the natural history or risk factors for cystic formation immediately following SRS. We aimed to address this gap in knowledge by reviewing our institutional experience. We reviewed our institutional record from 2007–2017 and identified 214 consecutive patients who underwent 1,106 SRS for BM and had >6 months of MRI follow-up. Demographic, clinical, dosimetric, and MRI data were reviewed. Statistical analysis was accomplished with Students t-test, univariate and multivariate logistic regression. The median age was 61 (range 19–91), with a median follow-up of 424 days (range 12–2,934 days). Eleven instances of cyst formation (0.9% of 1,106 treated lesions) were identified at SRS-treated BM sites among nine patients. Most cysts formed within a year of initial SRS. The median interval between first SRS and first evidence of cyst formation was 218 days. Seven of the nine patients (78%) with SRS-associated cysts suffered progressive cyst expansion and neurologic decline requiring steroid treatment. Four (57%) suffered continued neurologic decline despite steroid treatment and required surgical fenestration. On univariate analysis, receipt of >4 rounds of SRS to independent locations was the only variable associated with increased risk of cyst formation (odds ratio 16.58, p= 0.001). This association remained robust after adjusting for length of follow-up (odds ratio 13.59, p=0.003). This is the first study to assess cystic formation immediately following SRS for BM. The incidence of SRS-associated cyst formation during this time frame is significantly lower than previously reported for patients who survived >3 years (0.9% versus 8–10%). Cyst formation was a rare phenomenon except in patients who underwent >4 rounds of SRS, where one in three patients suffered this complication. A high proportion (78%) of SRS-associated cysts progressively expanded and required medical or surgical treatment.

CMET-24. NEXT-GENERATION EPIDERMAL GROWTH FACTOR RECEPTOR TYROSINE KINASE INHIBITORS FOR LEPTOMENINGEAL CARCINOMATOSIS

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EGFR mutation status is strongly correlated with leptomeningeal carcinomatosis in non-small-cell lung cancer. Historically, patients were treated with first-generation EGFR tyrosine kinase inhibitors, however most would eventually develop resistance and disease progression. Therefore, recent interest has sparked in investigating next-generation EGFR- TKI monotherapy. We report two patients treated with next-generation EGFR-TKI monotherapy, independent of whole brain radiotherapy, with favorable response and outcome. CASE 1: A 70-year-old woman with non-small cell lung adenocarcinoma in status post resection and erlotinib was in remission until 10 years later when she presented with findings of intracranial hypertension. Imaging demonstrated an enhancing right frontal lesion and leptomeningeal disease. After six months on afatinib 30 mg daily, she had near-complete resolution of symptoms and significant decrease in leptomeningeal enhancement. CASE 2: A 54-year-old woman with non-small-cell lung adenocarcinoma underwent resection and presented two years later with multifocal right frontotemporal hemorrhagic metastases and leptomeningeal enhancement. Pathology from brain tumor resection showed pulmonary adenocarcinoma with EGFR exon 19 mutation. She received a single pulsed dose of erlotinib 1500 mg followed by osimertinib 80 mg daily with significant improvement in symptoms and complete resolution on repeat neuroimaging two months later. DISCUSSION: Systemic chemotherapy alone has traditionally been ineffective in patients with CNS metastasis, likely due to poor penetration of the blood brain

barrier by early-generation EGFR-TKIs and evolving drug resistance. The next-generation EGFR-TKIs may have improved success in treatment of leptomeningeal metastases in non-small-cell lung adenocarcinoma when compared to the first- and second-generation EGFR-TKIs. This could be particularly true in patients with documented exon 19 deletions, as in our second case. CONCLUSION: Next-generation EGFR-TKI monotherapy could be considered in select patients with leptomeningeal metastases from lung adenocarcinoma. More research is needed to review this potential therapeutic option, especially for use as first-line therapy.

CMET-25. NEOPLASTIC MENINGITIS REGISTRY: USE OF MULTI-NATIONAL COLLABORATIVE EFFORTS TO UNCOVER TRENDS AND IMPROVE OUTCOMES IN LEPTOMENINGEAL DISEASE

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BACKGROUND: Neoplastic meningitis (NM) is a devastating complication of both hematologic and solid tumors, with a dismal prognosis. NM can present with a variety of symptoms, prognostic features are still debated, and treatment options vary based on disease status and differences in institutional protocols. AIM: NeMeRe: a Multi-Institutional Retrospective and Prospective Registry of Neoplastic Meningitis in Adults is an electronic clinical data registry that captures information regarding cancer history, diagnoses, treatments, and survival rates from patients with NM to provide an inclusive picture of disease status and management. METHODS: The registry was designed in REDCap by the Office of Patient Oriented-Research in the Penn State College of Medicine Department of Neurosurgery. Implemented in 2017, NeMeRe currently has nine international sites entering retrospective and prospective data, 480 patients currently enrolled, and new sites are continually applying for access. RESULTS: Data analysis reports are generated to explore the relationships between disease characteristics, comorbidities, and therapies, and enables researchers to investigate a large number of real-world diagnostic, prognostic, and therapeutic questions with greater agility and without the costs and inefficiencies of randomized controlled trials, which have proven challenging in this disease. By adding the features of data entry verification and propensity score matching, registry studies also provide results which are nearly as reliable as RCTs The dynamic nature of clinical registries also allows researchers to suggest edits to the database that reflect real time changes in practice. The authors will present several examples of such studies. CONCLUSION: NeMeRe is a database with the potential to answer critical questions about an increasingly common disease that has been very challenging to study using more traditional clinical trial paradigms.

CMET-26. PERIOPERATIVE IMAGING OF BRAIN METASTASES: A EUROPEAN ASSOCIATION OF NEURO-ONCOLOGY (EANO) YOUNGSTERS SURVEY

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BACKGROUND: Neurosurgical resection is an important treatment option in the multimodal therapy of brain metastases (BM). Perioperative imaging is established in primary brain tumors to assess the extent of resection. However, structured guidelines on the use of perioperative imaging for BM patients are so far missing. METHODS: The European Association of Neuro-Oncology (EANO) Youngsters committee designed a comprehensive questionnaire on the use of perioperative imaging. The survey was distributed to physicians with neuro-oncologic focus via the EANO and the European Association of Neurosurgical Societies (EANS) network. RESULTS: 120 physicians from non-European countries and European countries responded to the survey. 76/120 neurosurgeons, 18/120 radiation oncologists and 17/120 neurologists participated. 89/120 participants worked at academic hospitals and 39/40 participants worked in high patient volume centers as defined by >50 BM cases per year. Local standard operating procedures for perioperative imaging were applied by 94/120 physicians. The preferred preoperative imaging method represented MRI for 112/120 (93.3%) participants. Postsurgical imaging was routinely performed by 106/120 physicians. 77/120 participants indicated MRI as the preferred postoperative imaging method, however, only 71/120 performed postoperative MRI imaging within 72 hours after resection. No correlation of postsurgical MRI and localization at an academic hospital (58/79 [73.4%] vs. 19/27 [70.4%], p>0.05) or patient volume (49/71 [69%] vs 25/40 [62.5%], p>0.05) was evident. The most frequently indicated reason for postsurgical imaging was the assessment of extent of resection as participants indicated to adjust the radiotherapy plan or even considered re-surgery to achieve complete resection. CONCLUSIONS: This EANO survey indicates that preoperative MRI is the preferred imaging technique for the majority of physicians, whereas a high variability of postoperative neuroimaging routines including CT and MRI was observed. International guidelines for perioperative imaging with special focus on postoperative MRI are warranted in order to optimize perioperative treatment modalities for BM patients.

CMET-27. OUTCOMES OF LUNG CANCER PATIENTS WITH LEPTOMENINGEAL METASTASES IN THE TARGETED THERAPY ERA

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BACKGROUND: Improvements in detection and molecular characterization of leptomeningeal metastasis from lung cancer (LC-LM) coupled with cerebrospinal fluid (CSF)-penetrating targeted therapies have altered disease management. In this new era, outcomes of patients with LC-LM are not well-defined. This study identifies molecular and radiographic characteristics of LC-LM correlating with clinical outcome. METHODS: We retrospectively reviewed charts of 171 patients with LC-LM between 6/2009 and 6/2017 at Memorial Sloan-Kettering Cancer Center. Presence of targetable mutations (TM) was determined by targeted exome sequencing (MSKCC IMPACT). Radiographic involvement was scored by number of gadolinium-enhancing sites in eight locations. CSF studies included cytopathology, quantification of circulating tumor cells (CTCs), and cell free DNA (cfDNA) analysis. Kaplan-Meier survival curves were compared by log-rank analyses. RESULTS: Median overall survival after LC-LM diagnosis was 4.2 months; 84 patients (49%) harbored TM. Patients who received targeted therapy (Ttx) after LC-LM diagnosis demonstrated reduced hazard of death (HR:0.63; 95% CI:0.45–0.89; p=0.008). This trend was reversed for those receiving Ttx prior to LC-LM (HR:2.46; 95% CI:1.42–4.26; p-value: 0.001). A subset of 93 patients underwent MRI brain, spine and CSF cytology within 30 days of LC-LM diagnosis. Extent of radiographic involvement (3 or more sites vs. 2 or less sites) correlated with OS: (HR:1.56; 95% CI:0.96–2.54; p=0.075). Enumeration of CSF CTCs at diagnosis from 16 patients revealed that greater than 50 CTCs/3mL increased hazard of death (HR:3.66; 95% CI:1.195–11.22; p=0.02). Similarly, elevated ctDNA concentration in CSF was inversely correlated with survival in 21 patients (HR:2.74; 95% CI:1.01–7; p=0.02). CONCLUSIONS: In this largest study of LC-LM, presence of TM and Ttx for LC-LM was associated with improved survival. Extent of radiographic involvement and quantification of CSF CTC and cfDNA show promise as prognostic indicators. These findings support molecular characterization and CNS staging for clinical management, prognostication and clinical trial stratification of LC-LM.

CMET-28. IMPACT OF DISEASE SITE, SIZE AND SURGICAL RESECTION ON SURVIVAL FROM METASTATIC CNS NEUROBLASTOMA

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PURPOSE: To evaluate the impact of site of disease, size of the metastases, and impact of surgical resection of CNS metastases in patients with neuroblastoma. METHODS AND MATERIALS: Patients with neuroblastoma metastatic to the CNS were considered for surgical resection of the lesion(s) prior to undergoing conventional external beam radiation therapy (EBRT) and intraventricular radioimmunotherapy (cRIT). Lesions were classified as unifocal, multifocal, or leptomeningeal. Post-surgical EBRT consisted of 18 or 21 Gy craniospinal irradiation with a boost of

30–36 Gy to the metastatic site(s). A Mantel-Cox analysis was performed to assess the prognostic significance of disease site, size, and extent of resection on overall survival (OS). RESULTS: 93 patients with CNS neuroblastoma were assessed (2003–2017) with a follow-up of 24–177 (median 48) months since the detection of CNS disease. Lesions were unifocal parenchymal (N=54), leptomeningeal (N=6) or multifocal +/-leptomeningeal (N=33). As regards unifocal lesions, 40 (74%) were > 2 cm in largest diameter, and 44 (81%) underwent gross total resection (GTR). Following GTR, EBRT and cRIT, local control with no progression at the site was achieved in 39/44 (89%) patients. Systemic neuroblastoma in the absence of CNS progression occurred in 19/93 (20%). Improved OS was noted for those with unifocal vs multifocal disease (p=.01). Although a trend towards improved OS was associated with GTR, it was not statistically significant (p=0.12). Neither the size nor the site of the unifocal lesion impacted OS. CONCLUSION: Local control of the metastatic lesion is successfully achieved with the combined approach of surgical resection, EBRT and cRIT in 89% of patients with unifocal CNS neuroblastoma, with a trend towards improved OS in those undergoing GTR of metastases. There is no impact on the size or location of the metastatic lesion(s).

CMET-29. PRE-OPERATIVE DURAL CONTACT IS ASSOCIATED WITH SURGICAL CAVITY RECURRENCE AFTER POST-OPERATIVE STEREOTACTIC RADIOSURGERY

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BACKGROUND: Brain metastases can be expected in 20–40% of patients diagnosed with cancer. Resection of a solitary or symptomatic brain metastasis provides immediate decompression and has been shown to improve overall survival, generally with some form of adjuvant postoperative radiation to reduce risk of recurrence. We sought to evaluate risk factors for local recurrence after postoperative single-fraction stereotactic radiosurgery (SRS). METHODS: Patients who underwent surgical resection of a brain metastasis between 2006 and 2016 were retrospectively reviewed. Characteristics of the pre-surgical tumor, surgical resection, and post-surgical treatment were collected. Patients who received single fraction post-operative stereotactic radiosurgery (SRS) to the resection cavity were included for analysis. Surgical cavity recurrences were evaluated based on the location of their centroid within the dose distribution and categorized as infield, marginal, and out-of-field. RESULTS: A total of 58 patients with 60 resection cavities receiving post-operative SRS met the criteria for inclusion in study. During a median follow up of 20 months, 12 patients were noted to have surgical cavity recurrences with actuarial 1 and 2-year local failure rates of 15% and 18% respectively. Of the recurrences. 5 were infield, 5 were marginal, and 4 were out of field. Evaluation of the pre-operative tumor characteristics revealed that tumors with dural/meningeal contact had a significantly higher risk of local failure using Fishers exact test (p=0.025). Quadratic-mean-diameter, target volume, dose, and conformity index were not significantly associated with local recurrence. 1-year actuarial rate of adverse radiation effect (ARE) was 8% in this cohort. CONCLUSION: Dural/meningeal contact was associated with an increased risk of surgical cavity failure in patients undergoing post-operative SRS. In light of these results, and the recent consensus guidelines regarding contouring of post-operative surgical cavity for SRS, further consideration should be the addition of a dural margin in post-operative surgical cavity target delineation.

CMET-30. BRAIN METASTASES FROM EGFR-MUTATED NSCLC WHICH HAD ACQUIRED RESISTANCE TO EGFR-TKI. ~LESS-FREQUENT T790M AND PRESERVED RESPONSE TO OTHER TKIs~

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BACKGROUND: Despite the favorable response, most brain metastases (MBs) acquire resistance to TKIs. T790M is the most common mechanism and accounts for approximately half of acquired resistance to TKIs. The aim of this study is to clarify the role of T790M in the acquired resistance of BMs, and optimal treatment for BMs progressed after TKI. METHOD: Upfront TKI was performed for BMs from EGFR-mutated NSCLC, and TKI was changed at progression. Gefitinib, erlotinib, afatinib and osimertinib were used and the selection of these TKIs were owing to the physicians' decision. During the disease course, re-biopsies of progressed diseases were performed to verify mutations of EGFR, and the incidences of T790M were compared among the organs biopsied. The time to CNS-progression (TCP) were evaluated for each TKI. RESULTS: 141 cases were enrolled. Gefitinib was used only as the first line TKI (n=91). Erlotinib was selected for both the gefitinib-naïve patients (n=27) and after gefitinib (n=51). Afatinib (n=21) and osimertinib (n=17) were selected for only recurrent cases. TCPs after gefitnib, erlotinib, afatinib, and osimertinib were 13.4, 20.1, 19.9, and 13.8months, respectively. The history of treatment with gefitinib did not affect the TCP after erlotinib (HR:1.37, 95%CI:0.61–3.25, P=0.451). Re-evaluations of EGFR was performed using 107 samples (lung:51, serum:25, CNS:22, others:9) from 88 cases. The incidence of T790M from CNS samples was 9.1% (Tumor:1/6, CSF:1/16), and was significantly lower than that from the other organs (lung:50.1%, surum:28.0%, others:44.4%) (Odds ratio:0.130, P=0.001). The extremely low incidence of T790M and satisfied effects of TKIs even after TKI-failure suggested the different mechanism of acquired resistance of BMs to TKIs in compared with the extracranial lesions. CONCLUSIONS: T790M played only a limited role in acquired resistance of BMs to TKIs, and alterations of the types of TKIs were still recommended for the progressed BMs after TKI.

CMET-31. INTRACEREBRAL HEMORRHAGE FROM NEOPLASTIC ANEURYSM AS FIRST MANIFESTATION OF LUNG CANCER

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BACKGROUND: Metastatic cerebral aneurysms are an extremely rare complication of cancer. We present a case of an intracerebral hemorrhage (ICH) secondary to a neoplastic aneurysm as the initial manifestation of lung cancer. CASE REPORT: A 63-year-old man presented with acute aphasia and was found to have a left parieto-temporal ICH on brain imaging. Angiography demonstrated a fusiform dilation of the distal left middle cerebral artery suspicious for a mycotic aneurysm. Patient underwent hematoma evacuation and aneurysmal clipping; pathology showed intravascular atypical cells determined by immunohistochemistry to be an embolus from a primary lung cancer. Chest imaging revealed a previously undiagnosed lung nodule with hilar adenopathies. Needle biopsy of one of the lymph nodes demonstrated neoplastic cells identical to those visualized in the aneurysm, with final pathology consistent with poorly differentiated lung adenocarcinoma (EGFR, ALK and KRAS negative). Patient subsequently received Gamma Knife radiosurgery to the surgical bed, followed by carboplatin, pemetrexed and pembrolizumab for treatment of his systemic disease. DISCUSSION: Tumoral intracerebral aneurysms are rare, with about 100 cases published in the literature, the majority of them arising from cardiac myxoma or choriocarcinoma. Only six cases of neoplastic cerebral aneurysms from metastatic lung cancer have been reported, all presenting as ICH. Four of them died as a result of the hemorrhage, and the remaining two had complications that precluded the administration of further therapy, making ours the first case to receive cancer-directed treatment aimed at the aneurysmal metastatic lesion. CONCLUSION: Neoplastic cerebral aneurysms are rare, but should be considered in patients with malignancy presenting with a pattern of ICH suspicious for aneurysmal origin. There are no guidelines regarding the treatment of these uncommon aneurysms, but based on our case, we suggest approaching them as any other cerebral metastasis, with complete resection whenever possible, followed by stereotactic radiosurgery.

CMET-32. BILATERAL OCCIPITAL METASTASES: MANAGEMENT CONSIDERATIONS AND CORTICAL BLINDNESS

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INTRODUCTION: Treatment decisions for brain metastases balance potential benefits of tumor control, symptom alleviation, and survival, with the risk of functional impairment or reduced quality of life. Bilateral occipital metastases pose a risk of significant visual deficits and even cortical blindness, with an unclear rate of resolution and development of these debilitating symptoms following resection or radiotherapy. METHODS: We retrospectively reviewed all cases of bilateral occipital metastases treated with surgery and/or radiotherapy between 2008–2017 at the Brigham and Women's Hospital. RESULTS: 18 patients with bilateral occipital metastases (median age 64 years; 13 women, 5 men) were identified. The most frequent primary cancer sites were lung (56%), melanoma (17%) and breast (11%). Visual symptoms were present in 67% of all cases, of which 67% had a visual filed deficit, 17% had diplopia, and 17% had an acuity deficit. These metastases were initially managed with resection (44%), radiotherapy (28%), or both (28%). In patients who initially presented with visual symptoms, 75% improved with treatment while 25% remained stable. In those who presented with no visual symptoms, 67% remained at baseline, 17% worsened acutely, and 17% worsened permanently with treatment. The majority of patients were also managed with post-treatment steroids. A representative case illustration is discussed of a patient

who initially received whole brain radiation therapy (WBRT) for 10 days, with dexamethasone for the first 8 days of radiation. Upon completion of WBRT, the patient developed rapidly worsening visual acuity, which subsequently improved with bilateral occipital craniotomy for tumor resection. CONCLUSIONS: Patients with bilateral occipital metastases may present with visual symptoms, including visual field loss, reduced visual acuity, and diplopia. The management of bilateral occipital metastases involves consideration of symptomatology, disease burden, and goals of care. Visual deficits frequently improve following treatment but may also develop in a minority of patients.

CMET-35. DIFFUSION AND PERFUSION FEATURES OF METASTATIC BRAIN LESIONS

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PURPOSE: To determine clinically relevant MRI features of various metastatic brain lesions using classical and advanced post-processing techniques of both diffusion and perfusion-weighted MR images. METHODS: One hundred twenty-three brain metastases including breast (27), non-small cell lung cancer (NSCLC, 43) and other (44) were retrospectively assessed prior to radiation treatment with standard anatomical, diffusion and perfusion-weighted MRI. A total of 346 individual lesions were manually segmented. Complementary to tumor volume, apparent diffusion coefficients (ADC) and relative cerebral volume (rCBV) measurements, an independent component analysis (ICA) was performed with dynamic susceptibility contrast (DSC) in order to assess arterio-venous components and their overlap region relative to tumor volume and time to peak of T2* signal from each component. RESULTS: Results suggests that no differences are observable for either ADC or rCBV features (median value) across metastatic subtypes. Interestingly, ICA-derived arterial component was higher in breast and NSCLC compared to other patients, while the venous component was higher in breast compared to all other groups. No difference in the overlap component was observed between groups. Within the other group, we found that overlap has higher volume than venous and arterial components. For other group, the difference between arterial and venous components was as well significant. Median time to peak of arterial and venous components were 8.4s and 12.6s with no differences between lesion types. Additionally, the overlap component was positively related to rCBV in all groups. However, no correlation was found for arterial and venous components with respect to rCBV values. CONCLUSIONS: Advanced ICA-derived component analysis demonstrates perfusion differences in metastatic brain lesions not observable with classical ADC and optimized rCBV post-processing approaches.

CMET-36. BEVACIZUMAB WITH OR WITHOUT LOMUSTINE FOR METASTATIC ORBITAL, DURAL AND LEPTOMENINGEAL ESTHESIONEUROBLASTOMA

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Esthesioneuroblastoma is a malignant neuroectodermal tumor originating from the olfactory neuroepithelium. Bevacizumab is a specific inhibitor of the vascular endothelial growth factor ligand, preventing its interaction with receptors on endothelial cells. Lomustine is a widely used alkylating agent with good CNS penetration. We report 3 cases of metastatic dural esthesioneuroblastoma that responded to bevacizumab with or without lomustine. All patients had been treated up-front for Kadish Stage D disease with craniofacial resection, radiation therapy, and platinum-based chemotherapy with or without etoposide. One patient had been treated for recurrence with temozolomide plus sunitinib. Recurrent disease consisted of leptomeningeal, dural, orbital, and skull metastases. Patients 1 and 2 were treated with bevacizumab, 5 mg/kg every 2 weeks; patient 3 also received lomustine 110 mg/sq m every 42 days for 6 cycles. Two patients had partial responses and 1 had stable disease. Responses consisted of reduction in dural contrast enhancement, tumor cyst volumes, and perilesional edema, along with symptomatic improvement. Mean progression free survival was 388 days, and overall survival 432 days. These patients with metastatic orbital, dural and leptomeningeal esthesioneuroblastoma achieved durable responses with bevacizumab with or without CCNU. Antiangiogenic and alkylating agent strategies may play a role in salvage therapy for this tumor.

CMET-37. SUSTAINED CLINICAL AND RADIOGRAPHIC RESPONSE IN AN ADULT PATIENT WITH LEPTOMENINGEAL METASTASES FROM ACUTE MYELOID LEUKEMIA TREATED WITH INTRAVENTRICULAR METHOTREXATE

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Leptomeningeal involvement (LMI) in adult acute myeloid leukemia (AML) patients is rare. Treatment is not standardized and includes intrathecal chemotherapy, radiation therapy and systemic chemotherapy. We present the case of a 23 year-old female diagnosed with AML in June 2017 initially treated with idarubicin and cytarabine followed by hematopoietic stem cell transplant. The patient presented 6 months later with headache, nausea, diplopia and ataxia. MRI of the brain and entire spine showed leptomeningeal carcinomatosis. Patient was initially treated with systemic cytarabine and idarubicin. This was immediately followed by IT methotheraxate 12 mg via an Ommaya reservoir twice a week for 4 weeks. Clinical improvement was noted after only 2 IT treatments. Interval MRI showed complete response of the leptomeningeal disease after 4 weeks. Treatment was continued once a week for another 4 weeks followed by a repeat MRI confirming complete radiographic response. CSF cytology done before every intrathecal treatment did not show any leukemic cells. IT methotrexate was discontinued and patient is currently being followed in the clinic. This report documents excellent sustained clinical and radiologic response to IT treatment of a rare neuro-oncologic condition without any significant adverse effects.

CMET-38. IMPACT ON THE CLINICAL COURSE OF EGFR MUTATION ON BRAIN METASTASES FROM NON-SMALL-CELL LUNG CANCER FROM VIEWPOINT OF NEURO-ONCOLOGISTS

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OBJECTIVE: Molecular and genetic alternations of non-small-cell lung cancer (NSCLC) now plays an important role in patient care of this neoplasm. The authors focused on the impact of EGFR mutation status on brain metastases (BM) from NSCLC to better understand the most desirable management of BM from NSCLC. METHODS: This was a retrospective observational study analyzing 266 patients with BM from NSCLC diagnosed between January 2008 and December 2015 in our institute. EGFR mutation, overall survival (OS), durations from diagnosis to brain metastases, and related factors with OS were measured. RESULTS: Among 266 patients, 127 patients (47.7%) had EGFR mutations. EGFR-mutant (EGFR-mt), compared with EGFR wild-type (EGFR-wt), showed longer median OS from diagnosis (40 vs 21 months, $P < 0.001$) and after BM diagnosis (22 vs 11 months, $P < 0.001$) and higher frequency of BM, around 80% of which occurred within 2 years. Good prognostic factors for OS were positive EGFR mutation (Hazard Ratio (HR) 0.64), no BM at diagnosis (HR 0.56), and single brain metastasis (HR 0.65). Patients harboring EGFR-mt single brain metastasis showed longer OS and durations from 1st brain metastasis to 2nd brain metastases than patients with either multiple BM or EGFR-wt BM. For those without BM at diagnosis, EGFR mutation status did not have any impact on OS (40 for EGFR-mt vs 37 months for EGFR-wt). CONCLUSIONS: Our study is in agreement with other studies that EGFR mutation is associated with prognosis of patients with BM from NSCLC. Results of our study also suggest that careful observation or screening for BM is recommended especially with the first 2 years for NSCLC patients with EGFR mutation. Aggressive treatments for EGFR-mt NSCLC patients with single BM should be considered taking into account that these patients could show prolonged survival after BM.

CMET-39. INTRA-CSF LIPOSOMAL CYTARABINE PLUS SYSTEMIC THERAPY AS INITIAL TREATMENT OF BREAST CANCER LEPTOMENINGEAL METASTASIS: A RANDOMISED, OPEN-LABEL TRIAL

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BACKGROUND: Intra-cerebrospinal fluid (CSF) therapy for the treatment of leptomeningeal metastasis (LM). remains controversial. METHODS: DEPOSEIN (NCT01645839) was a multicenter randomized open-label study exploring the efficacy of liposomal cytarabine added to standard-of-care systemic therapy for the treatment of LM from breast cancer. Inclusion was based on the identification of tumor cells in the CSF or typical clinical and magnetic resonance imaging (MRI) signs of LM. Patients were randomly assigned to receive systemic therapy alone (arm A) or in combination with intra-CSF liposomal cytarabine (arm B). Neurological and patient-reported outcomes (PRO) were performed monthly, cerebrospinal MRI every 2 months. The primary endpoint was progression-free survival in the leptomeningeal compartment (LM-PFS); 66 events were required to ensure 80% power for a hazard ratio of 0.5 (two-sided alpha=5%). RESULTS: Thirty-seven patients were assigned to arm A, 36 patients to arm B. Baseline characteristics were similar between arms. The median number of liposomal cytarabine injections in arm B was 5 (range 1–20). Focal radiotherapy was performed in 6 (16%) and 5 (14%) patients in arms A and B, respectively. Serious adverse events were reported in 20 and 27 patients in arms A and B. In the intent-to-treat population, median LM-PFS locally assessed was 2.0 months (95% confidence interval (CI) 1.3 2.7) in arm A versus 4.3 months (95% CI 2.3 5.7) in arm B (HR=0.57, 95% CI 0.35 0.92, p=0.02). Sixty-eight patients have died. Median OS was 4.0 months (95% CI 2.2–6.5) in arm A versus 7.3 months (95%CI 3.9–12.6) in arm B (HR=0.80, 95% CI 0.50–1.29, p=0.35). Centrally reviewed LM-PFS and PRO will also be reported. CONCLUSIONS: The addition of liposomal cytarabine to systemic therapy may improve LM-related PFS but may not significantly improve survival. PRO will be essential to determine a possible clinical benefit from intrathecal chemotherapy.

CMET-40. LONG-LASTING RESPONSE IN SPINAL METASTASES FROM ALK REARRANGED NON-SMALL-CELL LUNG CANCER TREATED WITH DIFFERENT ALK INHIBITORS

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INTRODUCTION: About 40% of ALK-rearranged NSCLC patients develop brain metastases (BM), while leptomeningeal metastases (LM) occur in 5% of patients, and spinal intramedullary metastases in < 1%. Few data are available regarding the efficacy of ALK inhibitors in neoplastic spinal disease from NSCLC. CASE-REPORT: In March 2014 a 55 year-old woman developed multiple BM after 2 years from the diagnosis of an ALK-rearranged NSCLC who was receiving crizotinib. Crizotinib was continued associated with WBRT with a near-CR (RANO criteria) lasting 12 months. One year later a spinal MRI displayed multiple intramedullary enhancing lesions and diffuse leptomeningeal spread along the cauda equina, with CSF positivity for neoplastic cells. Ceritinib was started and a CR both on MRI and CSF was obtained lasting 18 months. In December 2017 the patient developed bladder dysfunction and paralytic ileus due to multiple intramedullary spinal and leptomeningeal recurrences. Considering the higher BBB penetration of lorlatinib, the patient started the drug and achieved a significant improvement of the urinary incontinence and intestinal transit after 3 months. Conversely, no change of the extent of spinal disease was observed on MRI. At this time, the patient is continuing treatment with lorlatinib and she is free of recurrence since 5 months. DISCUSSION: The development of CNS disease in ALK-rearranged NSCLC has been suggested to be a natural evolution of the disease and/or is correlated to low CNS penetration of the molecular drugs that control the systemic disease. The PROFILE trials have shown longer OS in patients with BM who received crizotinib beyond progression, but data are lacking on the activity of second- and third-generation ALK inhibitors. CONCLUSION: This is the first report of a prolonged clinical and radiological response using sequentially different ALK inhibitors in a patient with concurrent LM and spinal intramedullary metastases from an ALK-rearranged NSCLC.

CMET-41. LIFETIME LUNG, BREAST, AND SKIN CANCER BRAIN METASTASES INCIDENCE: A REPRODUCIBLE SEER-MEDICARE STUDY

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INTRODUCTION: With improvements in cancer detection and primary cancer control, brain metastases (BM) become a greater concern. The Surveillance, Epidemiology, and End-Results (SEER) program recently released data on BM diagnosed at the same time as primary cancer, referred to as synchronous BM (SBM). Tentative evidence of lifetime BM (LBM) is present in Medicare claims, expanding the utility of these new SBM data. PURPOSE: This is a reproducible SEER-Medicare study estimating synchronous versus lifetime frequencies of BM for primary lung, breast, and skin cancers. MATERIALS AND METHODS: SEER data were linked to Medicare claims from 2007–2014 to identify incidence proportions (IP) and average annual age-adjusted incidence rates of SBM and LBM. SEER SBM data were linked to Medicare claims and used as a gold standard to evaluate Medicare BM algorithms, the classification performance of which informed similar estimates of LBM incidence. RESULTS: The IP of SEER SBM in lung, breast, and skin cancers was 9.6%, 0.3%, and 1.1%, respectively. The greatest SBM IP among lung cancer patients was 13.4% for non-small cell lung cancer, and among breast cancer patients was 0.7% for triple-negative breast cancer. The greatest LBM IP among lung cancers was 21.7% in small-cell lung cancer, 4.0% in triple negative cases for breast cancer, and 1.7% in nevi and melanomas. Concordance between Medicare claims and SEER regarding SBM was 0.61 (95% CI: 0.60 - 0.62) for lung cancer, 0.45 (95% CI: 0.39 - 0.51) for breast cancers, and 0.65 (95% CI: 0.59 - 0.70) for melanoma. Fewer SBM cases were found in Medicare claims than in SEER. CONCLUSIONS: These analyses provide a population-level glimpse into the natural history of BM, estimating both synchronous and lifetime incidence in lung, breast, and skin cancers using a large dataset that is representative of the US.

CMET-42. BONE METASTASIS PREDICTS POOR PROGNOSIS OF PATIENTS WITH BRAIN METASTASES FROM COLORECTAL CARCINOMA POST AGGRESSIVE TREATMENT

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The presence of brain metastasis (BM) in patients with colorectal cancer (CRC) is usually associated with terminal stage illness; however, a subgroup of patients receiving aggressive treatment can have a satisfactory prognosis. This study was designed to investigate the profile of prognostic factors in CRC patients with BM treated aggressively. CRC patients with BM were retrospectively reviewed. Survival analysis was performed to identify potential prognostic factors in the entire cohort of patients and a subgroup of patients treated aggressively. Aggressive treatments included surgical resection, radiotherapy, and/or chemotherapy. Overall survival (OS) was defined as the time between the diagnosis of BM and death or until the date of the last follow-up visit. A total of 78 CRC patients were confirmed as having BM. Sixty-eight of them had extracranial metastases at the time of their BM diagnosis. The most common sites of extracranial metastases were lung (n=51, 65.4%), followed by liver (n=25, 32.1%), and bone (n=12, 15.4%). Fifty-one patients who were treated aggressively had significantly longer OS than those who accepted palliative care (14.1 months vs. 2.0 months, p<0.0001). Multivariate analysis was applied and results showed that aggressive treatment (n=51), recursive partitioning analysis (RPA) class I/II (HR=0.27, 95% CI: 0.12–0.6, p=0.001), and fewer BM (HR=0.4, 95% CI: 0.21–0.78, p=0.07) predicted longer survival. In contrast, the presence of bone metastasis, rather than lung or liver metastasis, at the time of diagnosis of BM (HR=2.38, 95% CI: 1.08–5.28, p=0.032) predicted a poor prognosis. Although the prognosis of CRC patients that have BM is frequently very poor, those with good performance status and few brain lesions responded to aggressive treatment, while those with bone metastasis at the time of diagnosis of BM had relatively dismal survival rates, even when treated aggressively.

CMET-44. PREDICTORS OF SURVIVAL IN NEURO-METASTATIC MERKEL CELL CARCINOMA

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BACKGROUND: Merkel cell carcinoma (MCC) is a rare cutaneous malignancy of neuroendocrine origin, with about 30 cases of brain metastasis (BM) reported in the literature. Historically, the treatment of neuro-metastatic MCC has largely included chemotherapy (CT) and radiotherapy (RT). The aim was to investigate predictors of overall survival (OS) in neuro-metastatic MCC. METHODS: In this retrospective study, we surveyed institutional databases and additionally conducted a systematic review of the literature to identify cases reporting on management of distant MCC BM. A pooled analysis was performed on the institutional and literature cases to assess predictors of OS. Survival analysis was done on R (ver 3.4.0) using a Log Rank statistic and cox proportional hazard

ratio. RESULTS: Forty cases were included for analysis, describing operative (14) and non-operative (26) management. Median time from initial MCC diagnosis to CNS involvement was 17.0-mos (IQR 10.5- 26.5), and most patients had a single BM (62.5%). Management of intracranial disease included RT (84.2%), systemic therapy (59.5%) and surgical resection (35%). Operative management was associated with a lower intracranial burden of disease (BoD, single BM: op 92.9% vs. non-op 46.2%, p=0.004), but similar systemic BoD. Median OS was longer in patients treated with neurosurgery (73-mos, 95%CI:31–115 vs. 25-mos, 95%CI:17–44, p ‹ 0.001). Both neurosurgery (HR 0.18, 95%CI:0.06–0.54, p=0.002) and having a single BM (multiple BM or leptomeningeal disease: HR 2.51, 95%CI:1.12–5.6, p=0.03) conferred an OS benefit on risk-unadjusted analysis. On multivariable analysis, only neurosurgical resection was an independent predictor of OS (HR 0.16, 95%CI:0.04–0.59, p=0.006), controlling for age, BoD and RT. Systemic therapy and RT were not associated with OS. CONCLUSIONS: Resection of MCC BM may confer a survival benefit relative to non-operative management given appropriate patient selection. Prospective investigation of multimodal management of neurometastatic MCC is warranted, especially given the promise of new immunotherapy agents in treating MCC.

CMET-45. CHECKPOINT BLOCKADE IMMUNOTHERAPIES FOR MELANOMA BRAIN METASTASES: IMPROVED SURVIVAL OUTCOMES IN A NATIONAL COHORT

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BACKGROUND: The recent successes of checkpoint blockade immunotherapy (CBI) and BRAFV600-targeted therapy trials have generated substantial promise for revolutionizing the management of patients with advanced melanoma. However, because early clinical trials of CBIs and BRAFV600-targeted therapy either excluded or included disproportionately fewer cases of melanoma brain metastases (MBM), the survival benefit of these novel therapies for MBM remains unknown. METHODS: The characteristics, management, and overall survival (OS) of patients who presented with cutaneous MBMs during 2010–2015 were evaluated using the National Cancer Database, which comprises 70% of all newly diagnosed U.S. cancers. OS was analyzed with risk-adjusted proportional hazards and compared by Kaplan-Meier techniques. RESULTS: 2,753 (36%) of patients presenting with stage 4 melanoma had MBMs. Following the 2011 FDA approvals for CBI and BRAFV600-targeted therapy, MBM patients demonstrated a 91% relative increase in 4-yr OS to 14.1% (95CI:12.2–16.1) from 7.4% pre-approval (95CI:5.3–10.0, p<0.001). Post-approval, the proportion of MBM patients that received CBI rose from 10.5% in 2011 to 34.0% in 2015 (p<0.001). MBM patients were more likely to receive CBI if they were younger, more recently diagnosed, had fewer comorbidities, insured privately or through Medicare, diagnosed in New England, had brain-directed RT, or had involvement of other metastatic sites. Initial CBI in MBM patients displayed a 2.4x improved median and 4-yr OS of 12.4 mos (95CI:10.4–15.8; vs. 5.2 mos, 95CI:4.7–5.9, p<0.001) and 28.1% (95CI:22.1–34.4; vs. 11.1%, 95CI:9.3–13.1), which persisted in multivariable analyses (HR 0.12, 95CI:0.03–0.49, p=0.003). These benefits were pronounced in MBM patients without extracranial metastases, in which CBI demonstrated improved median and 4-yr OS of 56.4 mos (95CI:25.0-NR; vs. 7.7 mos, 95CI:6.7–8.7, p<0.001) and 51.5% (95CI:38.9–62.8; vs. 16.9%, 95CI:13.5–20.6). CONCLUSION: Using a large national cohort composed of a "real-life" MBM treatment population, we demonstrate the dramatic OS improvements associated with novel checkpoint blockade immunotherapies.

CMET-46. INITIAL CLINICAL AND ADVANCED IMAGING OUTCOMES FROM A MULTI-INSTITUTIONAL PHASE I DOSE-ESCALATION TRIAL OF RRX-001 PLUS WHOLE BRAIN RADIATION FOR PATIENTS WITH BRAIN METASTASES

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BACKGROUND: RRx-001, a novel redox modulator was evaluated as a potential radiosensitizer with WBRT in a multicenter phase I trial for adults with brain metastases. The primary endpoint was to determine MTD. Secondary endpoints included intracranial response rate (IRR), survival and correlation of DCE-MRI parameters with outcome. METHODS: Dose-escalation was managed by a TITE-CRM design to establish MTD at a 20% estimated DLT rate with four planned dose levels (5, 8.4, 16.5, 27.5 mg/m2). RRx-001 was given pre-WBRT (30 Gy/10 fractions) then twice weekly during WBRT. Target accrual was 30 patients. Correlative DCE-MRI was performed in a subset of patients at baseline, 24 hours post-RRx pre-WBRT, 1 and 4 months. Linear mixed models were used to correlate baseline and change in 24-hour Vp (plasma volume) with change in tumor volume. RESULTS: Between 2/2015-2/2017, 31 patients were enrolled across 5 centers. Two patients dropped out at baseline, and seven were treated with RRx/concurrent temozolomide to be reported separately, for a total of 22 evaluable patients. Median age was 60 years (range 35–85), 13 were male, 55% had melanoma and 23% had NSCLC. No DLTs were observed within 28 days of treatment and 5 patients were treated at the highest dose level. IRR was 50%, five patients survived > 12 months, 3 were alive at last follow-up. Among 10 evaluable patients undergoing DCE-MRI with 64 total lesions at baseline, lower baseline Vp (p=0.002) and reduction in Vp at 24 hours (p=0.001) were associated with reduced tumor volume at 1 and 4 months. CONCLUSION: RRx-001+WBRT appears well-tolerated in adults with brain metastases, with several long-term survivors and IRR of 50%. Following a single dose of RRx, a reduction in Vp is associated with tumor response at 1 and 4 months, suggesting activity within tumor vasculature and utility as a biomarker of longer-term response.

CMET-47. CLINICAL EVALUATION OF FITNESS TO DRIVE IN PATIENTS WITH BRAIN METASTASES

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INTRODUCTION: Brain tumors can result in focal neurological and cognitive deficits which may impair the ability to drive. There are no evidence-based recommendations on driving restrictions for patients with brain metastases. Recommendations vary per practice, with extrapolation based on local driving and epilepsy laws. Occupational Therapy driving assessment (OTDA) may provide insight into limitations for this population. OBJECTIVE: To determine whether clinical neurologic examination is sufficient to predict suitability to drive in patients with brain metastases. METHODS: We assessed the concordance between Neurology assessment of suitability to drive (pass/fail) and OTDA in individuals with brain metastases. 40 subjects were prospectively enrolled. Neurooncology evaluation was performed as standard of care, including an interview and neurological examination. Subjects subsequently underwent OTDA during which a battery of objective measures of visual, cognitive and motor skills related to driving was administered. RESULTS: Preliminary results from the first 29 patients included are reported. Mean age was 68 years. Lung was the primary location of the tumor in 62% cases. More patients in the group that failed OTDA had bilateral brain metastasis (77.3% vs 42.9%,p=0.0478). The sensitivity of the Neurology assessment to predict driving fitness compared to OTDA was 22.7% and the specificity 71.4%. The 22 patients who failed OTDA were more likely to fail on Vision Coach (81.8%), MOCA (68.2%) and Trail Making (50%) tests. DISCUSSION: There was poor correlation between the assessment of suitability to drive by Neurology and the outcome of the OTDA in patients with brain metastases. Subtle deficits that may impair the ability to drive safely may not be evident on neurologic examination. The Vision Coach, MOCA and Trail Making tests were the most sensitive tests to predict driver safety. The results raise questions about the choice of assessments in making recommendations about fitness to drive in people with brain metastases.

COMPUTATIONAL OMICS

COMP-01. TRACKING THROUGH EDEMA: ENHANCED NEUROSURGICAL PLANNING USING ADVANCED DIFFUSION MODELING OF THE PERITUMORAL TISSUE MICROSTRUCTURE

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PURPOSE: Neurosurgical planning relies on diffusion tractography to model eloquent fibers. Peritumoral edema and neoplastic infiltration suppress the sensitivity to detect fiber tracts, particularly using deterministic

tracking. Confounding effects of tumor infiltration and edema results in significant discordance between subcortical, intraoperative electrical stimulation (IES) and preoperative fiber track visualization. We hypothesized that tracking in peritumoral zone, which includes vasogenic edema and neoplastic infiltration, could be enhanced by using a bi-compartment diffusion modeling. Due to the known tendency of primary malignant tumors, glioblastoma, to infiltrate surrounding white matter (WM), the bi-compartment model will be validated to distinguish peritumoral tissue in gliomas versus secondary brain tumors (metastases). METHODS: Patients with tumors (88 gliomas and 50 metastates) underwent 30 direction DTI and were fitted with standard tensor and *Fernet*, a free-water-invariant bi-compartment tensor model. Deterministic tractography was performed on each subject. Five WM tracts (corticospinal tract, inferior fronto-occipital, inferior longitudinal, arcuate and uncinate fasciculi) were extracted bilaterally in each patient using a shape-based clustering algorithm. For each subject, percentage change of edema volume was compared between standard and *Fernet* tensor models. RESULTS: *Fernet*-based tractography showed average increase in edema coverage of 87 +/- 25% (t=6.9, p CONCLUSIONS: Results show fiber tractography in peritumoral region is significantly improved with better diffusion modeling of peritumoral tissue microstructure. Additionally, difference in tracking between metastases and glioblastomas is representative of tracking being affected differentially due to infiltration in peritumoral regions. CLINICAL IMPLICATIONS: Our peritumoral tissue modeling incorporates edema and infiltration, leading to superior tracking, and hence robust surgical planning. In future, the peritumoral tissue maps could be used to elucidate differences in radiological diagnosis, surgical risk stratification, response to therapy, tumor invasion and tumor genetics, and neuroplasticity.

COMP-03. TUMOR CONNECTOME: INSIGHT INTO THE IMPACT OF CNS NEOPLASIA AND THERAPY ON THE BRAIN NETWORK

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PURPOSE: Although surgical planning tools are aimed at avoiding damage to eloquent tracts, resection as well as treatment like radiation, may alter the structural connectivity of the whole brain. This may lead to subtle cognitive deficits in the future. The aim of this work is to present a paradigm to create a Tumor Evaluation Connectome, which is a map of the brain that shows how the regions are connected to each other. Although, connectomes are routinely used in various studies these days, it is extremely challenging to create one in the presence of tumor or a resection cavity. Thus a tumor connectome will enable the quantitative evaluation of structural connectivity between brain regions. METHODS: The Tumor Evaluation Connectome was created as follows: the T1 images were parcellated into 153 anatomical regions, using multi-atlas tools. 119 gray matter regions were retained to build the connectome after the parcellations were manually assessed for mislabeling of unhealthy tissue. The DTI data was denoised, eddy and motion corrected. The anatomical labels in the T1 image were transferred to the DTI data, through a deformable registration method. 1 million streamlines were generated using deterministic tracking from mrtrix, seeded from voxels with fractional anisotropy exceeding 0.3. The Tumor Evaluation Connectome was defined with the 119 GM regions as nodes and the streamline count between them as the strength of the connection. These tumor connectomes will be used to generate network measures of deficit / change, as z-score from controls in the following pre-defined subnetworks: motor, visual, language, attention, memory, social cognition, cognitive control. CLINICAL IMPLICATIONS: Connectomes are an unmet need to refine tools for surgical planning, enhance recovery after brain tumor surgery, and assess the effects of therapeutic interventions is a specific, sensitive, and reproducible measure of the brain connectome.

COMP-04. R TOOLS FOR EXPLORATORY ANALYSIS OF PUBLICLY AVAILABLE DATA: HPAANALYZE AND BEYOND

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BACKGROUND: The Human Protein Atlas (HPA) is a comprehensive resource for exploration of human proteome which contains a vast amount of proteomics and transcriptomics data generated from antibody-based tissue micro-array profiling and RNA deep-sequencing. Data from the HPA are freely available via proteinatlas.org, allowing scientists to access and incorporate the data into their research. Previously, the R package *hpar* has been created for fast and easy programmatic access of HPA data. Here, we introduce *HPAanalyze*, an R package aims to simplify exploratory data analysis from those data, as well as provide other complementary functionality to *hpar*. RESULTS: *HPAanalyze* is an R package for retrieving and performing exploratory data analysis from HPA. It provides functionality for importing data tables and xml files from HPA, exporting and visualizing data, as well as download all staining images of interest. The package is free, open source, and available via Github. CONCLUSIONS: *HPAanalyze* intergrates into the R workflow via the *tidyverse* philosophy and data structures, and can be used in combination with Bioconductor packages for easy analysis of HPA data.

COMP-05. EVALUATION OF A DEEP LEARNING ARCHITECTURE FOR MRI PREDICTION OF IDH, 1p19q AND TERT IN GLIOMA PATIENTS

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Recent studies have highlighted the importance of using molecular biomarkers (IDH, 1p19q, TERT) to group gliomas that have similar clinical behavior, response to therapy, and outcome. An emerging hypothesis is that glioma specific genetic and/or molecular alterations manifest as specific observable changes in MR anatomical imaging. Morphologic and texture features, originating from anatomical MRI, have been investigated as imaging biomarkers to predict MGMT and glioma group status. These texture or morphologic based approaches pose several challenges including requirements for several preprocessing steps such as intensity standardization, skull stripping, and tumor segmentation. Deep learning is an important evolving technology in many different fields, including anatomic imaging, and can be used to empirically identify important features in a variety of modalities, including MRI. Importantly deep learning precludes the need for extensive pre-processing. We describe a convolutional neural network, evaluating resnet50, vgg16, inception and xception neural network architectures, that can predict IDH, 1p19q and TERT status utilizing conventional T2 weighted MRI imaging with intensity normalization and nonuniform intensity normalization (N4) bias corrections. The dataset consisted of 432 images (340 for training and 92 for validation) from patients published by Eckel-Passow et al *New England Journal of Medicine* (2015). The system achieved a weighted f1 score of 0.901, 0.937 and 0.924 for IDH, 1p19q and TERT prediction on the test dataset, respectively. IDH status was misclassified in 9 out of 92 patients, while 1p19q and TERT status was misclassified in 6 and 7 patients respectively. Our results demonstrate the potential of deep learning architectures applied to conventional MRI to predict molecular glioma groups.

COMP-06. GLIOBLASTOMA DEVELOPMENT MIRRORS THE DEVELOPING BRAIN

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Intratumoral and interpatient heterogeneity are characteristics of glioblastoma and constitute important challenges in overcoming treatment resistance and developing new, more effective therapies. Using single-cell RNA sequencing, we characterized 60 933 cells from 4 developing fetal brains and 8 glioblastomas. By using fetal brain development as a road map, we show a tri-lineage (astrocytic, oligodendrocytic, and neuronal) hierarchical organization in all glioblastomas. In each patient, a population of progenitor cancer cells was found at the apex of this hierarchy. These cells were enriched in our patient-derived glioma stem cell samples, and, like progenitors in the developing brain, were the main dividing cell population within the cancer. Using expression signatures obtained from single-cell RNA-sequencing, we isolated progenitor cancer cells and compared them to other glioblastoma cell types. We showed the progenitors are the most resistant to chemotherapy and the most tumorigenic in mouse xenograft models. This newly found conserved developmental organization points to the cell of origin and suggests new therapeutic approaches.

COMP-07. COMPARATIVE MOLECULAR LIFE HISTORY OF SPONTANEOUS CANINE AND HUMAN GLIOMA

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Sporadic glioma occurs in companion dogs at frequencies comparable to humans. Genomic characterization of canine glioma has a distinct merit, in that age distribution at the time of diagnosis implies a pediatric disease while the animals are in the adult stage of life. This creates an opportunity to compare relative timing of driver events to that of human glioma, and to evaluate the importance of host context in the presence of driver alterations. Second, breed-specific elevated cancer risk enables increased sensitivity to the characterization of evolutionary conserved mammalian mutational processes in gliomagenesis. We performed whole genome, exome, transcriptome and methylation sequencing on 188 canine tumor and germline samples. As in human adult gliomas, we found frequently occurring alterations in *Tp53* pathway, cell cycle pathway (*Cdk4*, *Cdkn2a*), and receptor tyrosine kinases (*Egfr*, *Pdgfrα*) in canine glioma. Common R132 mutations in the Idh1 gene reflected a remarkable and species-agnostic but cancer-specific driving effect. Frequent whole chromosome gains were observed in syntenic regions of chromosome 13, harboring *Pdgfrα* and *Myc*, but we found notable absence of 1p/19q co-deletion and *Tert* promoter mutations. We calculate mutational processes and highlight ones related to DNA damage repair and transcriptional strand bias in both species. We also estimate mutational rate and relative timing of mutations, and compare those to human glioma in mapping life history of glioma, i.e., are canine glioma more similar to adult or pediatric human glioma? We found coding mutation rate on the lower end of human adult glioma cases but with a broader spectrum (0.3–3 per MB), while subset of cases having mutations found in human pediatric cancer drivers, e.g., *Kmt2c*, *Setd2*, Bcor. In bringing together a large canine glioma genomic sequencing dataset and comparing to human glioma, our study provides unique insights into glioma etiology and the chronology of glioma-causing somatic alterations.

COMP-08. SURVIVING BY EXPLORING: COULD GLIOBLASTOMA CELLS FOLLOW THE THEORY OF EXPLORATORY ADAPTATION?

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BACKGROUND: Glioblastoma (GBM) prognosis remains abysmal despite decades of research. Standard approaches to treatment target the tumor growth mechanisms, and novel ones attempt to better recruit the immune system. However, cancer has a remarkable ability to adapt, surviving the introduced stress signals and thriving in new microenvironment settings. Understanding these fundamental processes of adaptation, and mechanisms specific to GBM, would enable a robust treatment approach that considers how cells utilize compensatory mechanisms. METHODS: We adapted a recent theory of exploratory adaptation -- prescribing how small random perturbations to the regulatory network could be propagated to changes in the cellular phenotype -- to examine the question of how GBM cells adapt across their microenvironment. We studied the transcriptomic profiles of spatially-separated tumor anatomical structures using patient-derived data from the IVY Glioblastoma Atlas Project. First, we asked how exploratory adaptation might explain the ability of cells to change their phenotype given their initial anatomical location; second, we tested if tumor cells from one location can adapt over time to resemble cells in neighboring locations. RESULTS: We defined the cellular phenotype at the functional pathway activity level and introduced notions of phenotype similarity and convergence. We simulated the dynamics of the pathway activities while cells attempt to obtain the phenotypes of cells from a different spatial environment. We estimated the distribution of each pathway activity over time, and observed some transitions (for example, the sulfur metabolism pathway shifting from a unimodal to a bimodal distribution) suggestive of exploratory adaptation. Several DNA repair pathways, including mismatch repair and non-homologous end-joining increased their distribution spread over time. Some pathways reached the tested destination distributions, but the global phenotypes did not meet our convergence criteria. CONCLUSION: Our results suggest that exploratory adaptation could partially explain adaptation in GBM, but could not fully account for it.

COMP-09. HFE EXPRESSION ALTERS OUTCOMES IN BRAIN TUMORS

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Iron homeostasis is essential for cellular energy production particularly in rapidly dividing cells. The HFE protein is critical for regulating cellular iron uptake. To explore the impact of HFE expression in glioblastoma (GBM) and other brain tumors, we utilized TCGA GBM and GBMLGG databases from GlioVis. Within the GBMLGG database which includes lower grade tumors, high expression of HFE was associated with significantly poorer survival. In GBM patients with high HFE expression survival was also significantly worse compared to low HFE expression. There was a strong positive correlation between high HFE expression and tumor grade, with the highest expression in GBM. HFE expression was also correlated to GBM subtype. The lowest expression was in the proneural subtype and the highest in the mesenchymal subtype consistent again with high expression associated with worse prognosis. Normally MGMT methylation is associated with better outcome. However, when combined with high HFE expression there is a significant reduction in survival time (12.9 vs. 20.8 months). To identify the possible molecular basis for these survival differences, we interrogated the reverse phase protein array data (RPPA) for TCGA datasets and discovered a correlation between high HFE expression and expression of a number of proteins. Annexin-1 has the highest correlation of expression with HFE. This protein plays a critical role in innate immune response through the generation of proinflammatory mediators and modulates migration and the phagocytotic ability of neutrophils and macrophages. We have previously shown that mutations in the HFE protein alter macrophage phenotype. Mutations in the HFE gene, the most common autosomal recessive polymorphism found in Caucasians, reportedly impacts the progression of a number of diseases including GBMs. This study identifies HFE as a negative modulator of outcome in brain tumors.

COMP-10. ANTI-CORRELATED TGFβ AND ALTERNATIVE END-JOINING DNA REPAIR SIGNATURES ASSOCIATE WITH OUTCOME IN PRIMARY GBM

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Transforming growth factor β (TGFβ), which is distinctively overexpressed in glioblastoma (GBM) while undetectable in normal brain tissue, could underlie GBM intrinsic radioresistance by endorsing effective DNA damage responses. We showed that most (5/7) primary GBM explants respond to TGFβ and are radiosensitized by TGFβ inhibition, but a few were unresponsive in both assays (*Neoplasia* 18:795–805). To further examine the link between TGFβ and the DNA damage response we used microarray transcriptomes from 369 IDH wild-type GBM patients from The Cancer Genome Atlas Consortium (TCGA). Surprisingly, our study unveiled a very strong inverse correlation (Pearson's correlation coefficient, PCC=-0.80; p<0.00001) between the single specimen gene set enrichment analysis (ssGSEA) using a signature for chronic TGFβ activity and one of alternative end-joining (alt-EJ), a backup pathway for DNA double-strand break repair that is error-prone and less effective. Notably, patients with the low TGFβ/high Alt-EJ profile exhibited significantly better progression-free survival (p<0.003) and overall survival (p<0.02). The same negative correlation of the TGFβ/Alt-EJ signature was evident in the GBM RNASeq TCGA data (PCC = -0.88, p<0.00001), which also showed that there was no correlation between TGFβ/alt-EJ and MGMT methylation status ssGSEA (p=0.8). These analyses are consistent with our hypothesis that high TGFβ in GBM opposes response to therapy and indicate that treatment with TGFβ inhibitors could potentially boost GBM therapeutic control. Moreover, since Alt-EJ depends on poly(ADP-ribose)polymerase 1 (PARP1), these data also suggest a novel mechanism by which to select patients that will be responsive to PARP inhibitors.

COMP-11. CHROMATIN RUN-ON AND SEQUENCING (ChRO-seq) PROVIDES RETROSPECTIVE MOLECULAR PROFILING AND TRANSCRIPTIONAL ACTIVITY OF BRAIN TUMOR SPECIMENS UNSUITABLE FOR CONVENTIONAL RNA SEQUENCING

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The human genome encodes a variety of poorly understood RNA species that play important roles in the etiology complex disease yet remain challenging to identify in clinical isolates. We recently developed chromatin run-on and sequencing (ChRO-seq), a novel technology that maps the location of RNA polymerase genome-wide using virtually any frozen tissue sample, including samples with degraded RNA that are intractable to conventional RNA-seq. Here we report an integrative analysis of ChRO-seq maps from 61 primary human glioblastoma (GBM) tumors with matching clinical data, obtained from a retrospective cohort of patients banked at SUNY Upstate Medical Center (Syracuse, NY) between 1987 and 2007 (characteristics: Male:Female ratio= 2:1; median age at diagnosis= 59 years; median KPS=80; median overall survival= 343 days). Using ChRO-seq we discovered the boundaries and expression levels of annotated and unknown genes, lincRNAs, and active distal enhancers. Surprisingly, active enhancers in primary GBMs closely resembled the patterns found in the normal human brain, suggesting that malignant GBM tissue remains similar to the cell of origin. Despite extensive overall similarity, approximately 12% of enhancers were unique to GBMs. These enhancers drive regulatory programs similar to those in the developing nervous system and are enriched for transcription factor binding sites that specify a stem-like cell fate. More importantly, we discovered a core group of immune-related transcription factors and their associated binding sites whose activity is highly predictive of clinical outcomes in primary GBMs (HR= 2.66; p = 3.8e-4). Finally, we used the characteristic signatures of cell-type-specific enhancer activation to deconvolve the proportion of immune cell types infiltrating each tumor. Our study uncovers new insights into the molecular etiology of GBM and introduces ChRO-seq as a useful approach to map regulatory programs contributing to complex and heterogeneous diseases.

COMP-12. TOWARDS BIG DATA IN DIGITAL NEUROPATHOLOGY WITH THE DIGITAL BRAIN TUMOR ATLAS

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Digital methods are increasingly applied to enhance disease classification, tissue segmentation, and prognostic profiling in virtually all medical specialties. They are recognized also for informing precision diagnostics in neuropathology. However, most computational algorithms depend on large training data sets, which are not yet readily available for tissue slides. Regarding brain tumors, only single histological repositories are freely available, which cover only few tumor types and are limited by poor tissue morphology. Thus, in order to fill this gap, we are in the process of establishing the 'Digital Brain Tumor Atlas', which will span the full diagnostic spectrum of brain tumors according to the latest WHO classification. The repository will be based on digitized slides from MedUni Vienna's neurobiobank, which is one of the largest in Europe containing well over 500,000 surgical samples from 1882 to 2018. We are currently scanning a total of 10,000 diagnostic H&E-stained brain tumor slides at high resolution using a Hamamatsu NanoZoomer XR scanner. To date, roughly 2,700 whole slide images that cover 102 different brain tumor types have already been digitized. All slides will be annotated with tumor type (including molecular subtype where applicable) and location as well as patient age and sex. We will put special focus on the group of diffuse gliomas, for which we have already scanned 500 slides, each providing expert segmentations for compact tumor, necrosis, hemorrhage, and preexisting brain tissue. In the future, this unique brain tumor slide repository will be made freely available online to enhance further research in digital neuropathology by 1, providing all data necessary for training meaningful machine learning algorithms and 2, comprising an independent dataset for external validation purposes.

COMP-13. SILENCE OF HIPPO PATHWAY INDUCES PRO-TUMORAL IMMUNITY: NEW THERAPEUTIC TARGET OF GLIOBLASTOMAS

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The Hippo pathway plays a critical role in various cancers, but its role in glioblastoma (GBM) has not been properly addressed. To assess the clinical relevance of the Hippo pathway in GBM, we generated a core gene expression signature reflecting silence of the Hippo pathway (SOH), and validate it in GBM patient samples from The Cancer Genome Atlas (TCGA) and in a separate cohort revealing that SOH signature was associated with poor prognosis in GBM in both cohorts. Expression levels of CTGF and CYR61, the most reliable and well-known downstream targets of YAP1 (a target of the Hippo pathway), were markedly increased in GBM with the SOH signature. GBM showing the SOH signature was associated with an increased Immune signature score (ISS), suggesting that these patients might be good potential candidates for immunotherapy. Genes differentially expressed between samples with the SOH signature and samples with an active Hippo signature showed that many inhibitory immune checkpoint genes, such as CTLA4, PD-1, and PD-L2, were upregulated in the SOH subgroup, suggesting that YAP1/TAZ may induce resistance of cancer cells to host immune response in GBM. Also, many M2-polarized microglial markers and innate immune pathways were activated in the SOH subgroup. The SOH signature was strongly correlated with the TGF-β signature, which was also associated with a high ISS and mesenchymal features. In summary, SOH induces protumoral immunity in various ways, which may be new immunotherapeutic targets of GBM.

COMP-14. TUMOR EVOLUTION DIRECTED GRAPHS IMPLY THERAPIES AGAINST MOVING TARGETS IN PAN-GLIOMAS

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Precision cancer medicine aims at defining targeted treatments based on personalized mutations. While this approach has been successfully applied to a few cancers, there are several obstacles in the adoption of these approaches in complex tumors such as the gliomas. Among the main challenges that preclude the efficacy of targeted therapies are clonal heterogeneity (different cancer cells within a tumor can present diverse genetic make-up), and the dynamic nature of tumors (tumors evolve to explore niches and escape therapies). To address this challenge and to predict the evolution of gliomas, we have applied the tumor evolution direct graph framework [1] to a longitudinal cohort of genomic data from 260 glioma patients. Since cancer is a dynamic process, the optimal treatment should not only consider present characteristics of cancer cells but also the evolutionary trends, which might eventually determine the clinical outcome. To this end, our framework assembles existing computational pipelines and machine-learning approaches to learn the patterns of tumor evolution and to assign the sequential order of key driving somatic alterations in glioma progression. In addition to our previous observations [2–3], this study found that *TERT* promotor mutations, chromosome 1p and 19q codeletion, *PDGFA* amplification, and *FGFR3-TACC3* fusion are all significantly early events. Meanwhile, not limited to hypermutation and mismatch repair protein alterations, a remarkable number of cases harbor tyrosine kinase mutations (such as *EGFR* and *MET*) at the late stage, highlighting the importance of targeting moving targets in treating recurrent gliomas. More importantly, the order of somatic mutations inferred from this longitudinal cohort was also used to preclude tumor behaviors based on early alterations, which provides the possibility of early-stage intervention based on moving targets. [1] Wang, *et al.* eLife, 3:e02869 (2014). [2] Wang, et al. Nature Genetics, 48:768–776 (2016). [3] Lee, Wang, et al. Nature Genetics, 49:594–599 (2017).

COMP-15. META-ANALYSIS OF CANCER CELL LINES BASED ON THEIR RESPONSE TO TUMOR TREATING FIELDS (TTFIELDS)

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Tumor Treating Fields (TTFields) therapy (low intensity, intermediate frequency (100–300 kHz alternating electric fields) is an approved modality for the treatment of glioblastoma. TTFields were shown to exert an inhibitory effect on numerous cancer cell lines with some variability in the response of different cell lines. The goal of the present study is to compare characteristics of cell lines based on their response pattern to TTFields. Forty different human cancerous cell lines were treated for 72 hours with TTFields at optimal cell-specific frequency at the same nominal intensity (1.7 V/cm). Cell survival and clonogenicity were determined. Functional analysis of differentially expressed genes and mutations associated with response to TTFields was performed based on the Cancer Cell Line Encyclopedia (CCLE) database. Sensitivity to TTFields was compared with pharmacological profiling (CCLE). TTFields application demonstrated varying degree of cytotoxic effect in all cell lines tested. The inhibitory response to TTFields was found to be distributed around an average of 50% with a cytotoxic effects ranging between 14% and 86% reductions in cell counts, and a clonogenic effect ranging between no effect and 88% reduction in the number of colonies. Pharmacological profiling based on IC50 values, revealed increased sensitivity to: Lapatinib, PHA-665752 and PLX-4720 within the group of cell lines which were less sensitive to TTFields. Functional analysis of cell line gene expression and mutation data revealed enriched pathways related to DNA damage repair response, cell migration, hypoxia signaling and oxidative stress. This meta-analysis

of cancerous cell line response to TTFields demonstrates the broad effectiveness of TTFields in various cell lines and define the optimal frequency to be applied for each. The data presented in this work suggest that beside their anti-mitotic properties, TTFields may have effects on other cellular pathways. Pharmacological profiling may offer a rational for combining specific agents with TTFields.

COMP-16. CORRELATING MGMT PROTEIN LEVEL WITH TEMOZOLOMIDE RESPONSE IN GLIOBLASTOMA PATIENTS USING MASS SPECTROMETRY

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Glioblastoma (GBM) is an aggressive primary brain tumor with median progression-free survival (PFS) of 7 months and median overall survival (OS) of 15 months. Standard treatment at diagnosis consists of surgery, radiation and temozolomide (TMZ). O-6-methylguanine DNA methyltransferase (MGMT) modulates TMZ effect and MGMT promoter methylation status is used to predict outcomes and guide treatment decisions. However, standard testing is limited by poor inter-assay reproducibility and a weak correlation between methylation status and MGMT expression. We quantitated MGMT protein by mass spectrometry in 32 GBM patients to assess its relationship to PFS and OS after upfront TMZ treatment. We obtained archived tumor samples from newly diagnosed GBM patients prior to treatment with surgery, radiation and TMZ. Tumor cells were microdissected and solubilized, and MGMT protein was quantitated using mass spectrometry. PFS (determined by RANO) and OS were assessed using the Kaplan-Meier method and log-rank test. Of the 32 patients (66% male; median age: 63 years),15 expressed MGMT protein. While methylation status agreed with absence of MGMT protein in 8/9 cases, 9/23 samples with unmethylated MGMT had undetectable MGMT protein, indicating poor agreement between methylation status and protein expression. Patients with MGMT levels below 150 amol/ug of total protein had longer PFS than those with higher MGMT levels (HR: 0.51; p=0.0399; median PFS: 303 vs. 260 days). Similarly, MGMT level below 150 amol/ug was associated with longer OS (HR: 0.49; p=0.0311; median OS: 659 vs. 534 days). Discordance between MGMT methylation status and protein expression was found in 31% of GBM samples. MGMT protein expression below 150 amol/ug correlated with longer PFS and OS in TMZ-treated patients. Mass spectrometry-based MGMT quantitation provides a direct readout of protein expression and may be more reliable than methylation testing.

COMP-17. BINDING FREE ENERGY ANALYSIS OF PROGRAMMED CELL DEATH PROTEIN PD1 TO ITS LIGAND PD-L1

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Therapies targeting immune checkpoints such as the programmed cell death protein receptor PD1 pathway represent a step forward for treatment of multiple solid cancers such as non-small cell lung cancer, metastatic melanoma, and renal cell carcinoma. Despite promising pre-clinical data, anti-PD1 monoclonal antibody nivolumab failed to reach its primary efficacy endpoint in a recent phase III randomized clinical trial, CheckMate-143. One possible barrier in glioblastoma is poor diffusion of large monoclonal antibodies across the blood-brain barrier. Identification of the amino acid residue hot spots in PD1 to PDL1 binding could help guide future efforts towards a small molecular inhibitor. We investigate the binding free energies involved in PD1-PDL1 complex formation with a molecular dynamics approach, and identify the specific amino acid sites important for PD1-PDL1 complex formation. Binding free energies were calculated by Molecular mechanics / generalized Born hydrophobic solvent accessible surface area (MM-GBSA) calculations. The residues on PD-L1 with the largest binding free energy delta between bound and unbound states include Arg 125, Tyr 123, and Arg 113 with -7.3 kcal/mol, -6.5 kcal/mol, and -4.3 kcal/mol respectively (with smaller contributions from Tyr 56, Met 115, Asp 122, Ala 121, Phe 19, and Ile 54), while those for PD1 are Glu 136, Gln 75, and Ile 134 with -5.9 kcal/mol, -5.3 kcal/mol, and -5.0 kcal/mol respectively (with smaller contributions from Ile 126, Tyr 68, Thr 76, Leu 128, Ala 132, and Asn 74). These residues define the core of the binding interface between PD1 and PD-L1.

COMP-18. MACHINE LEARNING DIFFERENTIATION BETWEEN PLEXIFORM NEUROFIBROMAS AND MALIGNANT NERVE SHEATH TUMORS IN PATIENTS WITH NEUROFIBROMATOSIS TYPE 1 (NF1) BASED ON MRI

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INTRODUCTION: Peripheral neurofibromas (NF) represent one of the most common clinical presentations of the NF1. About 10% of patients with NF1 develop malignant peripheral nerve-sheath tumours (MPNST), which is a major cause of morbidity in NF1 patients. Improved prognosis can be achieved if MPNST are diagnosed at an early stage permitting ablative surgery with wide resection margins. Better understanding regarding the natural history and biology of MPNST has been obtained in the last decade, however existing imaging modalities are still imperfect for diagnosis of MPNST. The aim of this study was to use a machine learning method in order to differentiate between benign plexiform neurofibromas from MPNST in patients with NF1 based on conventional MRI methods. METHODS: Among 23 NF1 patients with pathologically-defined MPNST treated in the last decade in TASMC, seven patients with available preoperative MRI studies were included in the study. For comparison, 7 patients with benign plexiform neurofibromas were chosen from the database of the Gilbert Israeli Neurofibromatosis Center. Regions of interest at tumor areas were extracted from the post contrast 2D T_1-weighted spin-echo images acquired on 1.5/3T MRI systems. For each patient, speeded-up-robust (SURF) features were extracted from lesion areas using Matlab bagOfFeatures algorithm. The algorithm was trained with number of visual words =500, grid point selection method, strongest features=0.8 and grid step size (in pixels)=[5 5]. The algorithm was tested with 100 iterations, with the data being randomly split into training (80%) and testing (20%) datasets. RESULTS: A code-book of lesion representations was generated for each group and was given as input to the support-vector-machine (SVM) classifier. Classification results achieved mean accuracy=80%, mean sensitivity=72% and mean specificity=87%. CONCLUSION: Our preliminary results suggest that a quantitative image representation method based on Bag-of-Features may assist in the classification between benign plexiform neurofibromas and MPNST in NF1.

COMP-19. WATER-CONTENT BASED ELECTRIC PROPERTY TOMOGRAPHY (wEPT) FOR MODELLING DELIVERY OF TUMOR TREATING FIELDS TO THE BRAIN

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BACKGROUND: Understanding how Tumor Treating Fields (TTFields) distribute in the brain is important for optimizing delivery. The distribution depends on the electric properties (EPs) of the brain, which are heterogeneous. Therefore, there is a need for methods that map electric properties within tissue with high spatial resolution. Water content based EP tomography (wEPT) utilizes the ratio of two T1w to map tissue water content, and EPs. wEPT has been applied to map EPs at 128 MHz. This study examines the suitability of wEPT to map EPs in the brain at 100–1000 kHz (TTFields frequency range). MATERIALS AND METHODS: A model connecting T1 images, Water content (WC) and EPs in the 100–1000 kHz range was created using tissue samples derived from bovine brains and aporcine CSF sample. For each sample, T1w images were acquired and WC and EPs measured. Curve fitting yielded models connecting Ir, WC and EPs. Next, wEPT was applied to map water content and EPs in tumor-bearing rat brains. EPs and WC were measured on 6 samples excised from each brain. The measured values were compared to the WC and EPs in the regions of the wEPT map corresponding to the sample. RESULTS: Water content estimated using wEPT agreed well with measurements on excised sample. A connection between EPs estimated with wEPT and the measured values was found. However, in some samples, especially samples excised from the tumors, large differences between wEPT-derived EP values and measurements existed. CONCLUSION: wEPT maps WC in healthy and tumor brain tissues However, the relationship between WC and EPs within this frequency range is not clear. Further work is required to refine this relationship enabling a robust non-invasive method for mapping electrical properties within the brain, and better strategies for optimizing TTFields treatment.

COMP-20. THE NON-INVASIVE DETECTION OF GLIOBLASTOMA-DERIVED CELL-FREE DNA IN PLASMA USING NEXT-GENERATION SEQUENCING AND AN UNTARGETED VARIANT SEARCH

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Detection of tumor-derived circulating cell-free DNA (ccfDNA) in plasma from glioblastoma patients remains elusive. The vast intra-tumor genetic heterogeneity of glioblastoma has limited targeted searches using *a priori* molecular profiling from a focal tissue sample. Recent data supports the isolation of shorter ccfDNA fragments relative to ccfDNA's principal mononucleosomal peak (< 150 bp vs. 167 bp, respectively) to both enrich for tumor-derived ccfDNA and reduce false positives associated with next-generation sequencing (NGS). Here, we sought to determine if size selection affords detection of glioblastoma-derived ccfDNA. The ccfDNA from 11 healthy controls and 10 glioblastoma patients was molecularly barcoded, PCR amplified, and short ccfDNA fragments (< 150 bp) isolated using an automated gel-based technology. Both the original and size-selected ccfDNA samples were capture enriched using a custom-designed, glioblastoma-targeted NGS panel (128 genes, 128 kb) followed by paired-end sequencing. A consensus sequence was determined for each group of PCR duplicates with an identical barcode. The number of PCR duplicates used to derive a consensus sequence has been termed family size (FS). Non-reference alleles (NRAs) from consensus sequences in exons were tabulated. In healthy controls, there was a 15-fold reduction ($P < 0.001$) in NRAs (i.e., false positives) in the short ccfDNA fraction at FS ≥ 20 compared to FS ≥ 1. At FS ≥ 20 in the short ccfDNA fraction, there were significantly more NRAs in glioblastoma patients (i.e., potential tumor-derived variants) compared to healthy controls (1,065 ± 406 vs. 174 ± 75 NRAs, respectively; $P < 0.001$). In glioblastoma patients, there were significantly more NRAs in the short ccfDNA fraction compared to the original unselected ccfDNA sample at FS ≥ 20 (1,065 ± 406 vs. 165 ± 135 NRAs, respectively; $P < 0.001$). Selection for short ccfDNA fragments coupled with molecular barcoding detects glioblastoma-derived ccfDNA through concomitant improvements in both sensitivity and specificity during untargeted searches employing panel-based NGS.

COMP-21. WHY DETAILS MATTER – THE ROLE OF ALTERNATIVE SPLICING IN GLIOBLASTOMAS

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An often-overlooked aspect of cancer biology is the alternative usage of different isoforms, often called isoform switches. Isoform switches give rise to proteins with different functionalities due to differences in, amongst others, protein domains. These switches are both frequent and important in development, homeostasis, and many cancer types. It is however currently unknown whether isoform switches play a role in glioblastoma (GBM), which is among the deadliest of solid cancer types. To investigate the role of isoform switches in GBM, we integrated the RNA-Sequencing data from The Cancer Genome Atlas (TCGA) and Genotype-Tissue Expression (GTEx) project creating the first large-scale isoform-resolution dataset containing normal brain tissue. Using this dataset, we identified hundreds of isoform switches with predicted functional consequences when comparing GBM and normal brain tissue. For a large fraction of GBM patients, isoform switches were more prevalent than deleterious mutations in cancer driver genes indicating their important role in gliomagenesis. This is further supported by the large number of isoform switches we find associated with poor survival outcomes of both GBM and other cancer types.

COMP-22. LARGE SCALE TRANSCRIPTOMIC ANALYSIS OF MELANOMA BRAIN METASTASES

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Brain metastases are the most common cause of adult brain tumors and highly challenging to treat. Additionally, melanoma brain metastases carry a particularly poor prognosis, with a median overall survival of 4–5 months. Standard-of-care treatment of brain metastases includes surgery, radiation, and chemotherapy. However, melanoma is a radio-resistant cancer, thus significantly limiting options for treatment of its metastasis to the brain. There is clearly a significant demand for new treatment approaches. We are conducting a large scale, whole transcriptomic analysis of melanoma brain metastases to provide insight into molecular changes that may contribute to metastasis as well as to identify potential therapeutic targets of the metastasized cancer. Total RNA was extracted from >50 melanoma brain metastases formalin-fixed paraffin-embedded (FFPE) samples and libraries constructed and enriched for coding region transcript fragments. Libraries were subjected to Transcriptome Capture (TCap) targeting 21,415 genes, which represents greater than 98% of the RefSeq exome, and sequenced using the Illumina HiSeq platform. Preliminary transcriptomic analysis of melanoma brain metastasis samples reveals that ion channel expression manifests as a prominent feature in metastatic melanoma and that in some cases correlates with poor clinical outcome. Greater than 20% of FDA approved drugs target ion channels. We report that repurposing of one such class of drugs targeting the ligand-gated neurotransmitter $GABA_A$ receptors can impair melanoma cell viability. We will report on differential expression analysis between recent melanoma transcriptomic studies[1,2] and melanoma brain metastases samples and correlation of expression with primary melanoma DNA variants as well as potential therapeutic targets based on anomalous expression of protein coding genes, including of ion channels. References: [1]Akbani, et al. (The Cancer Genome Atlas Network, TCGA). Genome classification of cutaneous melanoma. Cell 2015; 161(7): 1681–1696. [2]Tirosh, et al. Dissecting the multicellular ecosystem of metastatic melanoma by single-cell RNA-seq. Science 2016; 352 (6282): 189–196.

COMP-23. ASSESSMENT OF PITUITARY ADENOMA CONSISTENCY AND VASCULARITY USING TEXTURE ANALYSIS OF CONVENTIONAL MRI

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INTRODUCTION: Pituitary adenomas are the third most common primary brain tumor in adults, with an incidence of 73–94/100000 cases. Most adenomas can be effectively resected using endoscopic endonasal approach, yet in about 10% of cases the tumor may be fibrous requiring a more complex procedure. Preoperative assessment of pituitary adenoma consistency and vascularity is thus important for surgical planning and risk assessment. The aim of this study was to assess pituitary macroadenoma consistency and vascularity using texture analysis of conventional MRI. METHODS: Retrospective analysis of pre-operative MRI data of 109 patients with pituitary adenoma was performed. Tumor consistency (hard vs soft) and vascularity (vascular vs avascular) were determined intraoperatively by the surgeon. Radiomics analysis was performed on T_2-weighted (T_2W) and post contrast T_1-weighted (T_1W+C) images, and apparent diffusion coefficient (ADC) maps (available in 57 patients). Fifteen features were extracted from the lesion area for each modality, including: mean, minimum, maximum, median, percentile 10,25,75,90, standard deviation, kurtosis, skewness, entropy, root mean square (RMS), and energy. Between group differences were tested using independent samples T-Test. RESULTS: *Consistency:* Significant group differences ($p<0.05$) were detected for T_2W min and ADC mean, min, percentile 25,75 median and RMS values, with higher values detected for the soft tumors for all parameters, indicates lower tissue organization. *Vascularity:* Significant group differences ($p<0.05$) were detected for T_2W min, T_1W+C skewness and ADC mean, min, percentile 10,25,75,90 median RMS and energy values, with higher ADC and lower T_2W and T_1W+C values detected for the vascular tumors, indicating lower tissue organization and higher heterogeneity. CONCLUSION: Texture analysis of conventional MRI and particularly of ADC maps may assist in pre-operative assessment of hard and vascular pituitary adenoma, and may have important applications in the field of personalized medicine.

COMP-24. UTILIZING MACHINE LEARNING METHODS FOR SEGMENTATION AND CLASSIFICATION OF BRAIN TUMORS BASED ON CONVENTIONAL MRI

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INTRODUCTION: High grade brain tumors and brain metastasis are the two most common malignant brain tumors in adults. There is high clinical importance for differentiating between the two, as well as for sub-classification of metastases based on their origin, since medical staging, surgical planning, and therapeutic decisions are vastly different for each tumor type. MRI is the modality of choice for the assessment of patients with brain tumors. However, in many cases, differentiation between the tumor types may be challenging due to their similar appearance on MRI. The aim of this study was to develop an automatic method for segmentation and classification of brain tumors based on conventional MRI. METHODS: A retrospective analysis of 439 MRI data sets: 212 from patients with high grade

glioma and 227 from patients with brain metastasis of breast, lung and other origins, was performed. Data include post contrast 3D T1 weighted images acquired at 1.5/3.0 Tesla MRI and clinical information including age, weight and gender. Following preprocessing and automatic segmentation of the enhancing tumor, several features were extracted: bag of features, first and second order statistical, morphological and wavelet features. The classifiers were trained in a 5-fold manner on 80% of the dataset and tested on the remaining 20% of the dataset. Sensitivity, specificity and accuracy were calculated. RESULTS: Best classification results obtained when classifying between high grade brain tumors and brain metastasis, using quadratic SVM classifier with mean accuracy=0.85, sensitivity=0.82 and specificity=0.87. Classifying brain metastases by their origin achieved best results with cosin KNN classifier with mean specificity=0.85 but low sensitivity=0.46. CONCLUSION: The proposed study demonstrates the use of machine learning methods for automatic segmentation and classification of brain tumors based on conventional MRI. Such methods may have great clinical importance in assisting in the diagnosis of new patients.

DRUG DISCOVERY

DDIS-01. THE ANTIBODY-DRUG CONJUGATE ABT-414 DEMONSTRATES SINGLE-AGENT ANTI-CANCER ACTIVITY ACROSS A PANEL OF GBM PATIENT-DERIVED XENOGRAFTS

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ABT-414 is a novel antibody-drug conjugate (ADC) of monomethyl auristatin F (MMAF), a microtubule destabilizing agent, and an anti-EGFR antibody (ABT-806). ABT-414 has demonstrated promising efficacy in clinical trials against newly diagnosed and recurrent glioblastoma (GBM), but little is known about mechanisms of sensitivity and resistance to the drug. The efficacy of ABT-414 was evaluated *in vitro* across a panel of nine EGFR-amplified GBM patient-derived xenografts (PDXs). Six lines responded to therapy, with the EGFR vIII mutant lines (GBM6, GBM39, GBM76, GBM108) being more sensitive (IC_{50} ~0.01 ug/mL) than point mutant (GBM12) or wild-type (GBM84) amplified lines (IC_{50} ~0.5 ug/mL). In contrast, GBM26 (point mutant), GBM46 (vII mutant) and GBM59 (vIII mutant) were non-responsive (IC_{50} > 10 ug/mL). Further testing revealed that these latter three lines were also resistant to MMAE, a cell-permeable analog of MMAF, which suggests intrinsic resistance to the toxin. In the responsive lines, ABT-414 was further tested in tumors implanted in heterotopic and orthotopic locations using tumor volume measurements and/or bioluminescent imaging (BLI). In flank, GBM6, GBM39, GBM76 and GBM108 were all highly sensitive to therapy, with complete tumor regression observed in all mice extending beyond 200, 250, 130 and 50 days, respectively. Intracranial GBM39 tumors were also highly responsive, with continuous suppression of BLI signal to background, and a survival benefit greater than 155 days. In contrast, GBM6 was resistant to therapy, without detectable impact on tumor growth and a difference in median survival of 5 ± 10 days. GBM76 was intermediately sensitive, with an extension in median survival of 20 ± 10 days. In summary, the majority of EGFR amplified lines tested are sensitive to ABT-414 *in vitro* and as flank tumors, but efficacy in treatment of orthotopic tumors is more limited. We hypothesize this effect may be related to heterogeneity of drug delivery.

DDIS-02. NOVEL BISPECIFIC ACTIVATOR OF MACROPHAGES FOR THE TREATMENT OF GLIOBLASTOMA

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Patients undergoing current treatment for glioblastoma (GBM) still face a poor prognosis with a median survival of ~16 months. Current therapy is incapacitating and limited by non-specific toxicity to the surrounding brain. We have developed an immunotherapeutic approach that selectively targets GBM by redirecting the patients' own macrophages present within the natural glioma milieu (representing up to 30% of the tumor volume) towards the tumor in an antigen-specific manner. This novel molecule, called bispecific activator of macrophages (BAM), bridges the macrophages with the tumor cells through the binding of CD64 and EGFRvIII, a tumor-specific antigen, respectively. We generated a stable fully human EGFRvIII-specific BAM Chinese hamster ovary cell line, and confirmed expression of BAM by western blot. Binding to CD64 and EGFRvIII was confirmed by flow cytometry on human $CD64^+$ (hCD64) transgenic murine macrophages and $EGFRvIII^+$ gliomas. Simultaneous BAM engagement of hCD64 on macrophages, and EGFRvIII led to the induction of macrophage tumoricidal activity measured by reactive oxygen species release. Furthermore, *in vivo* BAMadministration into glioma bearing mice significantly reduced tumor growth. In conclusion, we have developed a novel immunotherapeutic approach capable of harnessing the body's own tumoricidal capacity by redirecting macrophages towards tumors via the administration of a bispecific activator of macrophages (BAM). While further studies are needed, this therapy offers promising results for the treatment of GBM.

DDIS-03. EGFR AMPLIFICATION INDUCED INCREASED DNA DAMAGE RESPONSE AND PREDICTED SELECTIVE SENSITIVITY TO TALAZOPARIB (PARP INHIBITOR) IN GLIOBLASTOMA STEM-LIKE CELLS

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Poly-ADP-ribose polymerase (PARP) is an enzyme critical for regulating a variety of DNA damage repair mechanisms. In this study, we report that PARP inhibitor, talazoparib, showed strong single-agent cytotoxicity and remarkable selective activity in glioma stem-like cells (GSCs). This single agent activity was strongly correlated with EGFR amplification as shown by genomic analysis. GSCs withEGFR amplification (which occurs in about 45% of GBMs) exhibited higher oxidative base damage, DNA breaks, and genomic instability than non-amplified GSCs. To sustain the elevated basal oxidative stress, EGFR amplified GSCs harbored increased basal expression of DNA repair proteins. As a result, DNA damage and PARP-DNA complexes increased in the amplified GSCs following talazoparib treatment, which may explain the sensitivity of these GSCs to talazoparib. Further, we show that EGFR kinase activity is important for talazoparib sensitivity, as kinase-inactive EGFR mutant and EGFR knockout cell lines were resistant to talazoparib. Intriguingly, another PAPR inhibitor Olaparib, with similar PARP enzymatic inhibition potential but less PARP-trapping ability compared with talazoparib, did not show selective sensitivity in EGFR amplified GSCs. Our data provide insight into the anti-cancer activity of talazoparib through PARP inhibition and by trapping PARP-DNA complexes. In conclusion we propose the potential of EGFR amplification as a biomarker for selection of the development of personalized therapy.

DDIS-04. COMPOUNDS IDENTIFIED BY STRUCTURE BASED VIRTUAL SCREENING DECREASE GBM BTIC GROWTH AND GLUCOSE UPTAKE

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Patient prognosis for individuals diagnosed with GBM remains incredibly poor with a median survival of only 15 months despite aggressive treatment with surgical tumor resection, chemotherapy, and radiation therapy. Therapeutic development to prolong survival has been hampered by a high degree of inter- and intra-tumoral heterogeneity. Contributing to tumor heterogeneity is a subset of highly tumorigenic cells, termed brain tumor initiating cells (BTICs), that can self-renew and have been shown to be highly invasive and resistant to therapy. BTICs can survive under nutrient poor conditions due to their increased expression of glucose transporter 3 (GLUT3). In a recently accepted study, we utilized structure based virtual screening (SBVS) using a GLUT3 homology model to identify two novel GLUT inhibitors. We are now generating a structure-activity relationship profile based on a highly efficacious compound (IC_{50}= ~300 nM) with the same backbone structure identified in the initial screen and seek to improve the potency, selectivity and drug-like properties of the GLUT inhibitors. We have tested several novel analogs and identified four that have maintained efficacy against BTICs *in vitro* (IC_{50}= 300–600 nM). Importantly, these analogs have displayed less toxicity to astrocytes than lead compounds in addition to improved stability in mouse liver microsomes. As proof of concept, we have begun to test the

GLUT inhibitors alone and in combination with chemotherapy *in vivo*. Thus far, our work has demonstrated that targeting glucose transport is a promising therapeutic avenue to explore.

DDIS-05. PATIENT DERIVED NEUROSPHERE CULTURES IDENTIFY NOVEL CHEMOVULNERABILITIES IN GLIOBLASTOMA

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3-dimensional (3D) cell culture systems are increasingly used in biomedical research due to their recapitulation of tumor architecture, including pertinent nutrient and oxygen gradients, and a requisite drug penetration through multiple cell layers. Deployment of patient derived models of cancer (PDMC) propagated in defined media (serum-free) heightens clinical relevance of drug activity over that of long-term established cell lines, with an expectation of greater preclinical prediction of efficacy in patients. Indeed 3D tumor models retain the genomic heterogeneity and "stem-like" properties of the original tumors. Here we report results from screening 3D neurosphere cultures of 24 different glioblastoma (GBM) PDMC with focused, clinically-relevant, drug collections. In addition to uncovering patient-specific agents (Precision Medicine), the approach uncovers substratification of GBM into novel chemosensitive groups. Specifically, cases dichotomize into instances of sensitivity to targeted agents such as proteasome inhibitors or those of growth factor signaling (e.g. TGFβ pathway modulators), although many novel targets are also identified. We further employ genomic and gene expression analyses to characterize said groups with a view to illustrating these chemovulnerabilities to detect the relevant signaling pathways' therapeutic intervention points. In sum, using clinically-relevant PDMCs and 3D culture systems, we can identify drugs and novel targets relevant to individual patients. Once further validated, genomic characterization of new patient cultures could be used to predict sensitivity to certain perturbagens, making a case for evidence-based precision medicine. As current GBM treatment options are particularly limited, this approach may help provide insights for optimal use of available therapeutics.

DDIS-06. AAV TOOLKIT ENABLING PRECISION COMBINATORIAL VIROTHERAPY FOR GLIOBLASTOMA

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BACKGROUND: Glioblastoma represents the most common and deadly type of brain tumor in adults. Although it is widely recognized that combinatorial therapeutic approaches may be necessary to achieve lasting remission or cure, identification of novel combinatorial therapies is challenging. Despite the availability of a number of potent and intuitively combined biologic monotherapies, the cost of performing studies using multiple biologic agents is prohibitive, and delivery of therapeutics within the brain is often difficult. Recombinant Adeno Associated Viral (rAAV) vectors have become the preferred vector for human gene therapy applications because of their flexibility, ease of production, and translatability from mouse to human studies. METHODS: We developed a method for rapid screening of cell lines for rAAV transduction efficiency using 29 different capsid serotypes. In addition, we created a large array of rAAV-encoded transgenes that are designed to enhance immune recognition or to have anti-proliferative, anti-invasive, or direct cytotoxic effects on brain tumor cells. The transgenes were engineered with a novel multimerization domain that increases expression several fold compared to unmodified transgenes. Our methods allow for high expression of transgene products that are secreted into the brain tumor microenvironment and act in a non cell-autonomous manner, while potentially avoiding the systemic toxicities associated with such therapeutic cocktails. RESULTS: We screened 14 primary human glioblastoma cell lines and several murine glioma cell lines, identifying AAV capsid serotypes that are capable of high efficiency transduction *in vitro*. This *in vitro* screen was reflective for the transduction of tumor and brain microenvironment seen by the serotypes *in vivo*. Efficacy studies of cocktails of anti-tumor transgenes delivered via rAAV *in vivo* are under way. CONCLUSION: We present the use of rAAV vectors encoding biologic agents as a technology accelerator for the preclinical identification of novel combinatorial therapy regimens for glioblastoma.

DDIS-07. MSC-DERIVED EXOSOMES AND microRNA IN GLIOMA THERAPY

Irtiza Hasan, Eric Momin, Joy Gumin, Satoshi Adachi, Brittany Kerrigan, Anwar Hossain and Frederick Lang; The University of Texas MD Anderson Cancer Center, Houston, TX, USA

Glioblastoma (GBM), the most common and deadly adult brain tumor, is notoriously resistant to therapy, not only because of the presence of Glioma Stem-like Cells (GSCs) but also because of the formidable delivery challenges imposed by the blood-brain barrier. Consequently, there is an urgent need to identify effective therapeutics and to elucidate successful strategies for delivering these new agents to GBMs. A promising new therapeutic approach is treatment with microRNAs (miRs), which are small, noncoding RNA that are powerful regulators of gene expression. We have shown that restoration of tumor suppressor miRs that are down-regulated in GBMs is capable of inhibiting the growth of GSCs. Despite the therapeutic potential of miRs, however, it is currently unknown which miRs will be most effective against GBMs and how these miRs will be delivered. To define miRs that are most effective against GBMs, we undertook a "candidate approach" based on a literature review and tested miR-124 & miR-128 as potential anti-glioma miRs. These initial analyses made it clear that a comprehensive evaluation of miRs that inhibit GBMs was lacking. Therefore, we have undertaken an unbiased high throughput screen of 578 miRs against a panel of patient-derived GSCs representing all TCGA-GBM classes. We identified 50 miRs that are highly effective at inhibiting GSCs regardless of TCGA subtype. Further, we demonstrated that exosomes derived from *ex-vivo* cultured mesenchymal stem cells (MSCs) can systemically deliver anti-glioma miRs to GBMs. Exosomes are naturally occurring nanovesicles that function as intercellular transport vehicles. MicroRNA-loaded exosomes (Exo-miRs) can be isolated and then used to deliver the miR to GBMs. Most importantly, we have shown that when systemically administered into mice, Exo-miRs "home" to brain tumors resulting in down-regulation of the miR target genes. These results suggest that MSC-derived exosomes can be used as systemic delivery vehicles of anti-glioma miRs.

DDIS-08. DEVELOPMENT OF BRAIN-PENETRANT EGFR INHIBITORS FOR GLIOBLASTOMA

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The epidermal growth factor receptor (EGFR) is altered in nearly 60% of glioblastoma (GBM) tumors, however, EGFR tyrosine kinase inhibitors (TKIs) have failed to improve outcomes for GBM patients. This can be attributed to the inability of conventional EGFR TKIs (e.g., erlotinib, gefitinib, lapatinib, afatinib) to effectively cross the blood-brain-barrier (BBB) and reach adequate pharmacological levels for a tumor response. For example, less than 10% of plasma drug concentrations are achieved in the brain for all FDA approved EGFR TKIs. Herein, we performed a structure-activity relationship (SAR) from the 4-anilinoquinazoline scaffold to improve brain penetrance by modifications of physiochemical properties amenable to BBB penetration. Our synthesis scheme aimed to develop EGFR TKIs with low molecular weight and polar surface area, few rotatable bonds and hydrogen bond donors and acceptors, while having high lipophilicity. This yielded novel EGFR TKIs with nanomolar potency against EGFR mutant, GBM patient-derived cells in culture, high BBB penetration (200% brain to plasma), and efficacy against patient-derived orthotopic GBM xenografts. Current efforts are aimed at improving metabolic stability and bioavailability to obtain a clinical EGFR TKI drug candidate for the treatment of GBM.

DDIS-09. IDENTIFICATION OF GSK3β INHIBITOR KENPAULLONE AS A TEMOZOLOMIDE ENHANCER AGAINST GLIOBLASTOMA

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Cancer stem cells are associated with chemoresistance and rapid recurrence of malignant tumors including glioblastoma (GBM). Although temozolomide (TMZ) is the most effective drug against GBM to date, GBM cells acquire resistance and become refractory to TMZ during treatment. Therefore, glioma stem cell (GSC)-targeted therapy and TMZ enhancing therapy may be effective approaches to improve prognosis of GBM. Many drugs

that suppress the signaling pathway related to maintain GSC or enhance the effect of TMZ have been reported. However, there are no established therapies. Here, we screened drug libraries that are composed of 1,301 existing drugs using cell viability assay using GSCs, then extracted Kenpaullone, a kinase inhibitor, as a TMZ enhancer targeting GSCs. Kenpaullone efficiently suppressed activity of glycogen synthase kinase (GSK)3β. Combination therapy of Kenpaullone and TMZ demonstrated suppression of stem cell properties and proliferation of both GSCs and glioma cell lines. Combination therapy for the mouse models significantly prolonged survival time compared with TMZ monotherapy. Taken together, Kenpaullone is a promising drug for the treatment of GBM by targeting GSCs and overcoming chemoresistance against TMZ.

DDIS-10. ENGINEERED EXOSOMES FOR GLIOMA THERAPY

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Glioblastoma (GBM) is the most common among all malignant brain tumors, with 17,000 diagnoses every year in the United States. Despite receiving surgery, radiotherapy, and chemotherapy, the median survival of GBM patients is only 14.6 months, and less than 10% of GBM patients survive more than 5 years. There is an urgent need for the development of more effective therapies. To this end, we have developed a novel method to deliver therapeutic antiglioma genes by exploiting exosomes derived from engineered HEK293 cells that can package plasmid DNA (pDNA), messenger RNA (mRNA) or microRNA (miRNA). These exosomes are modified using ligand display technologies to allow delivery of cargo to cell types of choice. For proof of concept we used the glioma cell line U87 containing a "floxed" dsR cassette upstream of a green fluorescent protein (eGFP) transgene; in the absence of the cre these cells express red fluorescent protein. Transfer of either cre expressing pDNA or mRNA using engineered exosomes induced cre recombinse-mediated excision of the floxed tdTomato cassette, and subsequent expression of eGFP *in vitro* as well as in *in vivo* glioma models. In addition to using engineered exosomes to deliver cre, we also successfully delivered both luciferase and cytosine deaminase-uracil phosphoribosyl transferase. This model provides a unique functional readout demonstrating that engineered exosomes can be used to deliver any functional genetic cargoes to the cell of choice. This technology allows the delivery of pDNA/mRNA/miRNA to treat GBMs, and has powerful implications for a broad range of biotherapeutics: cancer therapy, cancer vaccines or immunotherapeutics, and DNA editing (CRISPR/Cas9).

DDIS-11. A CK1a ACTIVATOR PENETRATES THE BRAIN, AND SHOWS EFFICACY AGAINST DRUG-RESISTANT METASTATIC MEDULLOBLASTOMA

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Although most children with medulloblastoma (MB) are cured of their disease, SONIC HEDGEHOG (SHH) subgroup MB driven by *TRP53* mutations is essentially lethal. Casein Kinase 1a (CK1a phosphorylates and destabilizes GLI transcription factors, thereby inhibiting the key effectors of SHH signaling. We therefore tested a second-generation CK1a activator against, *TRP53*mutant, *MYCN*amplified MB. Our novel CK1a activator inhibited SHH activity *in vitro*, acting downstream of the vismodegib target SMOOTHENED (SMO), and reduced the viability of sphere cultures derived from SHH MB. SSTC3 accumulated at high levels in the brain, inhibited growth of SHH MB tumors, and blocked metastases in a genetically-engineered vismodegib-resistant mouse model of SHH MB. Importantly, SSTC3 attenuated growth and metastasis of orthotopic patient-derived*TRP53* mutant, *MYCN* amplified, SHH subgroup MB xenografts, increasing overall survival. Thus, a CK1a agonist penetrates into the brain, and shows efficacy against metastatic *TRP53*mutant MB, which are resistant to existing therapies including the SMO inhibitors currently being evaluated clinically.

DDIS-12. ONC201: THE FIRST SELECTIVE, NON-COMPETITIVE DRD2/3 ANTAGONIST FOR CLINICAL NEURO-ONCOLOGY

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ONC201 has exhibited preliminary clinical evidence of anti-tumor activity and safety in high grade glioma patients. In this study, we identified and characterized a previously unknown binding target of ONC201. BANDIT – a machine learning-based target identification platform – predicted that ONC201 would bind with high selectivity to the G-protein coupled receptors (GPCRs) dopamine receptor D2(DRD2) and D3 (DRD3). DRD2 is overexpressed in glioma, controls pro-survival mechanisms, and its antagonism causes glioma apoptosis. β-arrestin and cAMP assays determined that ONC201 selectively antagonizes DRD2/3. Consistent with BANDIT and in contrast to antipsychotics that antagonize DRD2, ONC201 did not antagonize other dopamine receptors or other GPCRs. Schild analyses and radioligand competition assays revealed DRD2 antagonism at concentrations consistent with ONC201 anticancer activity. In accordance with superior selectivity, ONC201 exhibited a wider therapeutic window compared to antipsychotics. Combined DRD2/DRD1 inhibition was found to be inferior to DRD2 inhibition alone, suggesting that selectively targeting D2-like receptors yields superior anti-cancer efficacy. ONC201 exhibited a very slow association rate for DRD2 relative to antipsychotics, whereas the dissociation rate was similar to atypical antipsychotics that do not cause extrapyramidal symptoms. Alanine scanning mutagenesis of DRD2 identified 6 residues that are critical for ONC201-mediated antagonism of DRD2 and located in orthosteric and allosteric sites. Molecular docking revealed orthosteric interactions at TM-II and an allosteric interaction at the interface of TM-IV and –V that mediates the DRD2 homodimer interface. These orthosteric and allosteric interactions exhibited predicted affinities associated with the observed competitive and non-competitive effects, respectively, and point mutations at either site increased the Ki of ONC201 in competition assays with radiolabeled methyl-spiperone. In summary, we identify both non-competitive and competitive antagonism of DRD2 by ONC201 that may explain its unique selectivity, safety, and anti-cancer activity in clinical trials as the first compound to target this receptor for oncology.

DDIS-13. UNDERSTANDING GLIOBLASTOMA SUSCEPTIBILITY TO TOP2-TARGETING DRUGS FOR PERSONALIZED THERAPY

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Poor outcomes in glioblastoma are partially explained by unpredictable individual responses to therapy. Topoisomerase II (TOP2) poisons Etoposide and Doxorubicin, which induce double-strand DNA breaks, are effective for some glioblastoma lines. On the other hand, the TOP2-enzymatic inhibitor MST16 is used for lymphomas, but has not been tested for glioblastoma. We investigated biomarkers for glioblastoma susceptibility to these drugs to repurpose them with a precision medicine approach. We performed a genome-scale CRISPR knockout (KO) screen on an Etoposide-susceptible cell line using this drug to select resistant clones. The screen identified 106 genes from DNA damage response, a pathway that was over-represented among genes involved in etoposide susceptibility. We overlapped genes whose loss confers Etoposide resistance from our screen with genes whose expression correlates with susceptibility to this drug across 35 glioma cell lines. This approach elucidated 6 genes, including some that regulate protein synthesis, as TOP2 poison biomarker candidates. Validation through CRISPR KO of several of these confirmed that their loss confers resistance to Etoposide and Doxorubicin, and their protein levels were predictive for TOP2 poison susceptibility across glioma cell lines. In contrast, these biomarkers were not predictive of glioma response to TOP2-inhibitor drug MST-16. Through ChIP-seq, we discovered that TOP2B binding coincides

with open chromatin in promoters of PDGFRA. Moreover, TOP2 inhibition with MST-16 downregulated this gene. Baseline expression of PDGFRA across glioma cell lines was highly predictive of MST-16 susceptibility, which was confirmed in an independent dataset of patient-derived glioma xenograft lines. Using complementary unbiased approaches, we identified several novel biomarkers for personalizing TOP2-targeting therapy for this disease. Inter-individual differences in glioblastoma susceptibility to TOP2 poisons relates to protein synthesis and DNA damage, whereas susceptibility to TOP2 inhibitors relates to TOP2B-mediated transcriptional regulation of an oncogene.

DDIS-14. MODIFIED CARBAZOLES DESTABILIZE MICROTUBULES AND KILL GLIOBLASTOMA MULTIFORM CELLS

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Small molecules that target microtubules (MTs) represent promising therapeutics to treat certain types of cancer, including glioblastoma multiform (GBM). We synthesized modified carbazoles and evaluated their antitumor activity in GBM cells in culture. Modified carbazoles with an ethyl moiety linked to the nitrogen of the carbazole and a carbonyl moiety linked to distinct biaromatic rings exhibited remarkably different killing activities in human GBM cell lines and patient-derived GBM cells, with IC50 values from 67 to > 10,000 nM. Measures of the activity of modified carbazoles with tubulin and microtubules coupled to molecular docking studies show that these compounds bind to the colchicine site of tubulin in a unique low interaction space that inhibits tubulin assembly. The modified carbazoles reported here represent novel chemical tools to better understand how small molecules disrupt MT functions and kill devastating cancers such as GBM.

DDIS-15. A NOVEL DOPAMINE RECEPTOR 3 ANTAGONIST INHIBITS THE GROWTH OF PRIMARY AND TEMOZOLOMIDE RESISTANT GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is the most common, lethal primary adult brain tumor with patient survival of only 14 months. The location and invasion of GBM leads to rapid recurrence after therapy. The standard of care chemotherapy is DNA damaging agent temozolomide (TMZ), to which resistance is common and is due, partly, to expression of the DNA repair enzyme O-6-methylguanine-DNA methyltransferase(MGMT), regulated by promoter methylation. To improve treatment of GBMs, including those resistant to TMZ, we explored targeting dopamine receptor signaling. Prior reports indicated roles for dopamine receptor 2 and 4 in GBM, with these inhibitors being effective in combination with EGFR inhibitors or temozolomide, respectively. We demonstrate that dopamine receptor 3 (DRD3) is an alternative target for therapy, with an expected low risk of severe side effects due to restricted expression in non-tumor brain. Six novel antagonists of DRD3 decreased the growth of GBM xenograft-derived neurosphere cultures, with minimal toxicity against human astrocytes and neurons. For those compounds, with a potential therapeutic window, two (SRI-21979 and SRI-30052) readily crossed the BBB and yielded no signs of liver or kidney dysfunction. In orthotopic models, 10 mg/kg of SRI-21979 per day for ten days, alone or combined with TMZ, trends toward increased survival. In striking contrast, we observed no benefit for haloperidol treatment in combination with TMZ beyond that for TMZ alone. Further analysis of TCGA data demonstrated that, unlike DRD2 and DRD4, DRD3 levels were not reduced in MGMT unmethylated GBMs and higher levels of DRD3 were associated with worse prognosis, suggesting that DRD3 antagonists may remain efficacious in TMZ resistant GBMs. SRI-21979, but not haloperidol or TMZ, significantly reduced the growth of TMZ resistant U251 cells and neurospheres derived from a TMZ-resistant xenograft. Our data demonstrate that DRD3 antagonist based combinatorial therapies may provide a potential, novel therapeutic treatment for GBM.

DDIS-16. ONC201 IN COMBINATION WITH RADIATION EXHIBITS SYNERGISTIC EFFICACY IN HIGH GRADE GLIOMAS AND OTHER ADVANCED CANCERS

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INTRODUCTION: ONC201 is the first small molecule DRD2 antagonist for oncology that is being investigated as a single agent in advanced cancer clinical trials. Downstream of DRD2 antagonism, ONC201 activates the integrated stress response pathway and apoptosis. ONC201 has exhibited preclinical and clinical anti-tumor activity in high grade gliomas. Given that ONC201 exhibits broad synergy with anti-cancer drugs, excellent safety, and single agent activity in tumor types where radiation is used routinely, we evaluated the combination of ONC201 with radiation in solid tumors. METHODS: Cell viability was evaluated in human and/or mouse breast, prostate and high-grade glioma cell lines in response to ONC201 (1 -10uM), radiation (2– 10Gy), or the combination. Incubation times ranged from 24 to 96 hours and the sequence of the two agents in combination was varied. RESULTS: Cell viability assays for ONC201 in combination with radiation in breast or prostate cancer cell lines revealed a cytotoxic response to the combination than was superior to either single agent. Western blot analysis of PC3 cells showed a synergistic induction of CHOP and ATF6 that are components of the integrated stress response. In MDA-MB-468 cells, Western blot analysis demonstrated a striking induction of PARP cleavage, a marker of caspase-mediated apoptosis, with 2 μM ONC201 in combination with 2 Gy radiation, whereas either single agent produced minimal PARP cleavage. In 4T1 murine triple-negative breast cancer subcutaneous tumors, the combination of oral ONC201 and radiation produced antitumor effects at subtherapeutic doses. In diffuse intrinsic pontine glioma cell lines, combination indices computed from cell viability experiments indicated modest synergy (~0.7 CI) for the combination ranging between 1–5 μM ONC201 and 2–10 Gy. CONCLUSION: ONC201 combines synergistically with radiation in high grade gliomas and other solid tumors.

DDIS-17. MULTI-LEVEL DRUG DEVELOPMENT PIPELINE FOR THE DISCOVERY OF TUMOR MICROTUBE TARGETING DRUGS

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Tumor microtubes (TMs), which are ultra-thin and long cellular protrusions extended by glioma cells, have emerged as a novel mechanism of glioma cell growth, invasion and interconnection with consecutive therapy resistance. So far, few molecular drivers of TM formation and function (Gap43, Cx43, Ttyh1 and actin dynamics) have been identified and their downregulation strongly reduced therapy resistance and glioma cell dissemination *in vivo*. We implemented a multi-level screening pipeline which allows to test drugs for their anti-TM actions. Initially drugs are tested for their general actions on glioma cell growth *in vitro* before being evaluated by laser scanning microscopy for their impact on TM formation (number of TMs), morphometry (TM length and diameter) and functionality (calcium communication) in 2D models. These parameters as well as anti-invasive properties are further validated in a 3D matrix model, which has shown to correlate well with the phenotype observed *in vivo*. As a final step of the *in vitro* pipeline, we developed a brain organoid model with patient-derived glioma cells, which is used to confirm the observed effects of the most promising compounds in a more complex microenvironment. Effective drugs identified in the *in vitro* screen are then tested in our mouse model *in vivo*: tumor growth, TM formation (number, morphology, interconnection) and function (calcium communication, invasion, therapy resistance) are longitudinally followed on a single cell level using a chronic cranial window and intravital two photon microscopy. Finally, classical drug effectivity parameters such as survival and tumor size on 7-T MRI scans are measured. First results of compounds, which have demonstrated tumor microtube-inhibiting vs. -promoting effects in this pipeline will be presented. In summary, this novel pipeline enables the rapid development of anti-TM drugs to translate the growing evidence of the importance of TMs to glioma biology into clinical trials.

DDIS-18. THE PROTEASOME: TARGETING THE NF-κB PATHWAY IN THE TREATMENT OF GLIOBLASTOMA MULTIFORME

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Glioblastoma Multiforme is a rare neoplastic disease of the brain that is known for its rapid aggressive growth and its poor clinical outcomes secondary to limitations in treatment efficacy. As such, the discovery of new agents to hinder and slow the proliferation of GBM is paramount for the eventual eradication of the disease. One such pathway of targeting

is the proteasome, as it provides a unique and novel means of targeting the growth and metastasis of aggressive forms of GBM. Expression of the proteasome is integral for the canonical NF-kB pathway, an inflammation pathway heavily associated with the progression of numerous cancers. One means of activation of the stress induced pathway is via increase pressure. As neoplasias of the brain grow within the confined space of the skull, they press against their stroma and experience higher interstitial pressure, further activating an NFkB mediated positive feedback cascade. By inhibiting the proteasome with novel imodazoline complexes, maintenance of the NFkB pathway is blocked and pressure stimulated growth should be halted. Stimulation of cultured U87 glioblastoma cells with 15mgHg for various periods of time induced a pressure activated increase in cell population, as well as activation of the NFkB pathway as well as in HEK293 embryonic kidney cells. Using our TCH-013 proteasomal inhibitor, pressure induced proliferation of these U87 cells appeared to be inhibited. Furthermore, using our TCH-01 compound pressure induced activation of the NFkB pathway was greatly in the HEK cells, indicating our imadazoline complexes target the pressure induced pathway at a downstream site enough to occlude the effect. Further screening of these compounds effects on pressure stimulated activation of NFkB and proteasomal activity is necessary in further brain cancer cell types.

DDIS-19. CT-179: AN INHIBITOR OF THE OLIG2 TRANSCRIPTION FACTOR WITH POTENT ANTI-TUMOUR ACTIVITY IN BRAIN CANCER

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High Grade Glioma (HGG) is incurable and has a median survival of less than 5% at five years, highlighting a desperate need for new therapeutic strategies. OLIG2 is a basic helix-loop-helix (bHLH) transcription factor that is expressed in neural progenitor cells during embryonic development where it sustains their replication-competent state and regulates their oligodendrocyte and motor neuron multi-lineage potential. In HGG, OLIG2 is re-expressed at high levels and drives an oncogenic program that leads to dysregulation of the cell cycle and subsequent gliomagenesis. This central role for OLIG2 in HGG initiation and growth, along with its low expression in normal tissues, identifies OLIG2 as a target for HGG therapy. We report the characterisation of an orally bioavailable small-molecule OLIG2 inhibitor, CT-179, the first bHLH transcription factor targeting drug developed for the treatment of cancer. The drug is well tolerated and easily penetrates the blood brain barrier, where it reduces brain tumour burden in orthotopic mouse and zebrafish avatar models. Mechanistically, CT-179 displayed nanomolar anti-proliferative activity and induced significant apoptosis mediated through disruption of the cell cycle that resulted in mitotic catastrophe at prometaphase. CT-179 showed enhanced anti-tumour activity in mouse models of HGG when used in combination with standard of care radiotherapy and temozolomide. These studies demonstrate that the pharmacological inhibition of OLIG2 is an effective treatment strategy for HGG that warrants rapid translation into the clinic.

DDIS-20. NEW A6K BORON DRUG DELIVERY SYSTEM FOR CLINICAL APPLICATION OF BORON NEUTRON CAPTURE THERAPY (BNCT)

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In BNCT in GBM, one of the keys to success can depend on the boron compounds. The combination of BSH and BPA in clinical GBM BNCT showed good results and that meant the multi boron use in BNCT was one answer to next step of BNCT. In this time, we showed that the new self-assembling peptide DDS with BSH toward clinical application of BNCT. The self-assembling A6K peptide was found and reported by Dr. Shuguang Zhang, MIT in 1982. The A6K peptide showed self-assembling feature in water, and worked as drug delivery system of siRNA with only mixture. The A6K drug delivery system was clinically approved to breast cancer trial in Japan since 2015. We observed the complex of A6K and BSH with scanning electron microscope in different mixture ratio. Next, we checked the cell toxicity, measured intracellular boron concentration and observed BSH localization in mouse model. At first, we established the simple A6K/BSH complex making method, as just mixture the BSH and A6K water solution by itself. The BSH/A6K complex with different mixture ratio showed different shape and different diameter of complex in SEM image. The ideal range of particle size of DDS is 20nm to 200 nm, and ours' complex diameter was about 40nm. Next, we administrated BSH/A6K complex to GBM cells and measured intracellular boron uptake. The concentration with BSH/A6K complex in U87 delta EGFR was 10 times or higher than that with BSH. Finally, we administrated BSH or A6K/BSH complex through mouse tail vein and got brain tumor sample after 12hr. The A6K/BSH mouse brain sample showed specifically accumulated BSH in tumor area. A6K peptide is clinical use in DDS and will spread various drug delivery tool for various clinical fields in future. Our A6K/BSH complex is very promising boron drug for next generation BNCT.

DDIS-21. KIF11 INHIBITORS FILANESIB AND ISPINESIB AS NOVEL AGENTS FOR MENINGIOMA THERAPY

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KIF11 is a highly conserved motor protein, which plays an important role in cell division by separating the spindle poles. KIF11 have been reported to be involved in multiple cancer types including melanoma, retinoblastoma, and glioma. In our previous studies, we found grade-specific expression levels of KIF11 in meningioma. Higher expression of KIF11 was associated with shorter progression-free survival and siRNA mediated knockdown inhibited the proliferation of two different meningioma cell lines in vitro. KIF11 inhibitors like Filanesib and Ispinesib are available and currently tested in phase II clinical trials for treatment of multiple myeloma. We investigated their potential benefit for treating meningiomas. To evaluate the potential of Filanesib and Ispinesib on meningioma cell lines, crystal violett assay was performed. Dose-curve analysis revealed IC50 values of less than 1 nM in the benign meningioma cell line Ben-Men-1 and the anaplastic cell line NCH93. Proliferation assay showed that single shot inhibitor dosage of 10 nM and higher led to an average inhibition of Ben-Men-1 cells by 58% and 66% on day 1 and 2 for Filanesib and by 63% and 60% for Ispinesib. NCH93 cells decreased their proliferation by 63% and 84% for Filanesib and by 67% and 89% for Ispinesib ($p<.001$). To explore the mechanism of diminished proliferation, we performed a FACS analysis. It revealed a G2/M block after treatment with Filanesib or Ispinesib compared to control in both cell lines ($p<.001$). Since KIF11 inhibition in other tumors have shown reduced cell migration, we performed a scratch assay. Treatment with KIF11 inhibitors resulted in reduced migration only in NCH93 cells compared to control after 8 and 12 hours by 20% and 85% ($p<.05$). Currently, we are testing KIF11 inhibitors in a xenograft mouse model. Taken together, KIF11 inhibitors Filanesib and Ispinesib could play a potential role in future meningioma therapy.

DDIS-22. WSD0922: A BBB PENETRABLE EGFR/EGFRVIII SMALL MOLECULE FOR THE TREATMENT OF GBM AND METASTATIC CNS TUMOR

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Aberrant EGFR signaling caused by mechanisms of overexpression, mutation, or autocrine activation contributes for oncogenesis by inducing cell proliferation and resisting apoptosis. In patients with GBM, an overall frequency of 25–64% mutation is EGFRvIII (exon2-7 deletion). In patients with NSCLC harboring active EGFRm+(Del19 and L858R), up to 65% brain/leptomeningeal metastasis develop ultimately after EGFR TKIs treatment. Current approved EGFR TKIs have demonstrated limited therapeutic efficacy against CNS tumors due to insufficient BBB penetration and/or poor activity against EGFRm+ such as EGFRVIII. The occurrence of GBM and rate of CNS metastases of NSCLC are increasing, thus there is a high unmet medical need for the development of a BBB penetrable EGFR inhibitor (common tumor drivers in the brain: EGFRVIII/Del19/L858R) to clinic. Herein, we report a discovery of brain penetrable EGFR inhibitor WSD0922 for GBM and NSCLC CNS metastasis with IC_{50} against EGFRm+ ex-vivo GI_{50} in GBM tissues with EGFRvIII is In-vitro MDCKII transfected cells and Caco-2 assays have shown that WSD0922 is highly permeable ($61x10^{-6}$ cm/s) and not a substrate of P-gp or BCRP, two main efflux transporters expressed on human BBB. Preclinical CNS PK studies confirmed good brain penetration of WSD0922 with $K_{p,uu,brain}$ close to unity. Significant TGI or regression for mice bearing GBM PDX model with EGFRVIII was achieved by treatment with WSD0922. Moreover, treatment of mice bearing PC-9 (Del19 in NSCLC) in both subcutaneous and intracranial models with WSD0922 resulted in dose dependent TGI or regression with statistically significant survival benefit. Predicted human PK properties are very promising to offer sufficient target engagement in clinic. Taken together, our data provide a good rationale for WSD0922 to be developed toward clinic for the treatment of patients with GBM and CNS metastasis of NSCLC harboring EGFRm+.

DDIS-23. BETULINIC ACID SUPPRESS GLIOBLASTOMA CELLS THROUGH INHIBITION OF UNFOLDED PROTEIN RESPONSE

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OBJECTIVE: The current anti-neoplastic drug for glioblastoma multiforme (GBM) is limited because of insufficient blood-brain-barrier (BBB) penetration and development of resistance. Betulinic acid (BA) is a natural product of plant origin, has been reported to induce Sp1 degradation against temozolomide (TMZ)-resistant GBM and capacity of BBB passage. In this study, we investigated the alternative anti-neoplastic mechanism of BA. MATERIALS AND METHODS: To explore the mechanism of tumor inhibition by BA in TMZ-resistant cells and uncover novel therapeutic targets for recurrent GBMs, we used high density oligonucleotide microarrays to analyze gene expression during BA treatment. Furthermore, we applied Ingenuity Pathway Analysis (IPA) software performing Canonical Pathways Analysis. We subsequently examined the protein expression of genes identified from the microarray data. RESULTS: We identified the unfolded protein response (UPR), known as a signaling network activated by endoplasmic reticulum (ER) stress functioning to alleviate this stress, appeared most significantly affected by BA. Protein expressions of ER stress-related factors were observed with different concentration of BA. CONCLUSION: BA is able to inhibit the survival of TMZ-resistant GBM cells via inhibition of unfolded protein response. BA is a good therapeutic strategy to overcome the tumor recurrence after the initial therapy. Key words: Betulinic acid, temozolomide resistance, glioblastoma, unfolded protein response.

DDIS-24. PROTEASOME INHIBITION IS A TARGETED THERAPY FOR PTEN-DEFICIENT GLIOBLASTOMAS

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One of the most common genetic alterations in glioblastoma (30–40%) occurs in the *PTEN* (phosphatase and tensin homolog) tumor suppressor gene, where loss of function has been mechanistically linked to increased tumor cell invasion, and to a lack of radio- and chemo-therapy response. To identify new drug compounds that target PTEN-deficient brain tumors we performed a high throughput drug screen using patient-derived GBM spheres and found that PTEN-deficient samples were highly sensitive to proteasome inhibition. We confirmed this sensitivity in GBM spheres and iNPCs (inducible Neuronal Progenitor Cells) by genetically over-expressing or deleting PTEN, where PTEN over-expression decreased and PTEN-deletion increased sensitivity to the drug, respectively. Additionally, proteasome inhibition specifically suppressed tumor growth in mice of orthotopically engrafted human glioblastoma samples. Mechanistically, we determined that PTEN-deficient cells are more sensitive to proteasome inhibition due to an increase in protein synthesis rate and loss of autophagy activity associated with activation of the PI3K/mTOR pathway. This study reveals that proteasome inhibition is a targeted-therapeutic strategy for PTEN-deficient brain cancer.

DDIS-25. TARGETING GLIOBLASTOMA HETEROGENEITY WITH miR-34a

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microRNA-34a could serve as a novel therapeutic agent as it is underexpressed in Glioblastoma and modulates the expression of multiple genes in the deregulated p53, Rb and receptor tyrosine kinase networks which confer selective growth advantage and represent significant intra-tumoral heterogeneity, a major cause of therapeutic resistance. We studied the effects of microRNA-34a transfection in three primary patient-derived lines (GBM 6, GBM118 and GBM 126, respectively belonging to classical, mesenchymal and proneural subtypes), four established cell lines (T98G, U251, A172, LN229; where T98G and U251 show primary resistance to treatment while A172 and LN229 are sensitive) and two cell lines with acquired resistance to temozolomide (A172-TR, LN229-TR). Transfection with microRNA-34a mimics significantly reduced proliferation and sensitized to temozolomide (Combination Index< 0.2–0.6) and radiation (dose enhancement factor 1.7–2.2) treatment, regardless of baseline treatment resistance in all studied cell lines. We identified broadly conserved binding sites in the 3'UTR of multiple mRNAs in the Glioblastoma deregulated networks and genes known to confer therapeutic resistance and validated the direct downregulation of Bcl-2 protein as a major contributor to temozolomide sensitization. For in vivo delivery, nanocells (400 nm diameter), termed EDV, were derived from genetically modified bacteria, provided with a bispecific antibody targeting EGFR and loaded with microRNA-34a. EDVs were injected intravenously while temozolomide was administered by oral gavage in GBM6 orthotopic mouse model. We observed a significant reduction in tumor growth in mice treated with microRNA-34a EDV relative to control EDV-treated mice (p=0.021). Further, microRNA-34a EDV significantly improved survival and synergized with temozolomide therapy [p<0.001, median survival of control EDV, microRNA-34a EDV, control EDV with temozolomide and microRNA-34a EDV with temozolomide was 44, 48, 86 and 147+ days respectively]. In conclusion, microRNA-34a EDV is a promising novel therapeutic that inhibits Glioblastoma tumor growth and counteracts therapeutic resistance and intra-tumor heterogeneity.

DDIS-26. BTP-7, A NOVEL PEPTIDE FOR THERAPEUTIC TARGETING OF MALIGNANT BRAIN TUMORS

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High-grade gliomas are deadly cancers, and current standard-of-care has demonstrated limited success. The ability to specifically target glioma cells can allow for the development of safer and more efficacious brain cancer therapy strategies. Brevican, a CNS-specific extracellular matrix protein is upregulated in glioma cells and its expression correlates with tumor progression. Particularly, a brevican isoform lacking glycosylation, B/bΔg is a unique glioma marker and not expressed in non-cancerous tissues. Therefore, B/bΔg represents a valuable target for anti-cancer strategies. Here, we describe the utilization of state-of-the-art platforms to screen a one-bead-one-compound combinatorial peptide library to discover a novel "B/bΔg-Targeting Peptides", called BTP-7 that can bind B/bΔg with high affinity and specificity. BTP-7 displayed 260 nanomolar affinity for recombinant B/bΔg protein, and had little association with the fully glycosylated isoform of brevican (control). Scrambling of the BTP-7 sequence led to complete abrogation of B/bΔg binding. Furthermore, BTP-7 is preferentially taken up by B/bΔg-expressing glioma cells compared with non-expressing cells. We also discovered that BTP-7 can cross the blood-brain barrier using both the *in vitro* BBB organoid model and in mice. BTP-7 displayed 10x greater binding to intracranial GBM-6 tumors than control peptides, and 4x higher tumor uptake than in normal brain tissues. Conjugation of BTP-7 to camptothecin (an anti-tumor drug) via a cleavable linker led to increased DNA damage in intracranial GBM-6 tumors and prolonged survival in tumor-bearing mice. Our results show the potential of BTP-7 for the development of next-generation targeted therapeutics that could greatly benefit the outcome of patients with advanced brain cancer.

DDIS-27. COMBINED INHIBITION OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) AND POLY (ADP-RIBOSE) POLYMERASE (PARP) IMPAIRS GLIOBLASTOMA CELL GROWTH

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BACKGROUND: Glioblastoma is aggressive with poor prognosis. Nicotinamide phosphoribosyltransferase (NAMPT) is essential to maintain nicotinamide adenine dinucleotide metabolism during rapid proliferation and regulates poly (ADP-ribose) polymerase (PARP), which is crucial for DNA repair. Targeting both NAMPT and PARP may represent a treatment strategy in glioblastoma. We hypothesize that the combined inhibition of NAMPT and PARP can induce synergistic cell death in tumor, while sparing significant cytotoxicity in normal astrocytes. METHODS: NAMPT expression was determined in a group of six human glioblastoma cell lines and normal human astrocytes (NHA) by Western blotting. To analyze the cytotoxic effects of the treatments in tumor and normal cells, U251 and NHA cells were selected to receive FK866 (NAMPT inhibitor), Olaparib (PARP inhibitor), or both drugs for 72 hours prior to the cell viability test. RESULTS: Various levels of NAMPT expression were demonstrated in a group of glioblastoma cell lines and NHA, where U251 showed the strongest expression. We demonstrated a significant decrease of cell viability in U251 cells that were treated with FK866 in a dose-dependent manner. A 32% reduction of cell viability was demonstrated at a dose as low as 10 nM. However, a 28% reduction of cell viability was found in NHA at

the EC_{50} concentration for U251 cells. When tumor cells were treated with a combination of FK866 and Olaparib at their EC_{50} concentrations (28nM and 406nM), 72% and 50% reductions of cell viability occurred in tumor cells and NHA, respectively. CONCLUSION: The combined treatment with FK866 and Olaparib enhances cytotoxicity in glioblastoma cells compared to the single-agent therapy, representing a promising therapeutic strategy. However, the toxic effects on NHA are concerning and warrant further investigations to determine a precise therapeutic window in preclinical models before the treatment is considered for clinical trials.

DDIS-28. GENE THERAPY WITH oHSV-1 CD/5-FC EXHIBIT ENHANCED ANTITUMOR EFFICACY IN GLIOMA CELL LINE AND INTRACRANIAL MURINE GLIOMA MODEL

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BACKGROUND: Glioblastoma is one of the most common and lethal types of primary brain tumor. The current standard therapy for GBM consists of surgical followed by combination of radiotherapy and chemotherapy with TMZ. However, median survival following surgery, radiotherapy and TMZ remains dismal. Thus, there is an urgent demand to develop novel, effective therapies for this malignancy. Oncolytic herpes simplex virus 1 (oHSV-1) was one of the first genetically-engineered oncolytic virus. After modifications, oHSV-1 selectively replicated in and killed tumor cells while sparing normal cells. In our study, oHSV was armed with the suicide gene cytosine deaminase (CD) and then we test the efficacy of oHSV-CD/5-FC in glioma cell lines and intracranial murine glioma model. METHODS: First, we performed MTT assay to inspect the oncolytic activity of oHSV-CD/5-FC in glioma cell lines. U87-Luc cells were implanted stereotactically into the striatum to generate intracranial tumors. 18 days after tumor implantation, mice were randomly divided into groups and intratumorally (IT) injected (same stereotactic coordinates) with oHSV-CD or PBS. 5-FC was administered 3 times intraperitoneally (i.p.). Tumour growth was monitored via bioluminescent imaging using IVIS Spectrum system and magnetic resonance imaging (MRI) once a week after virus injection. RESULTS: We treated four cell lines and two primary cultured GBM cells in a 48-hour MTT assay. All cell lines tested showed sensitivity to oHSV-CD. IC_{50} ranged from 0.01 (U87 MG) to 0.12 (U251 MG) MOI. And together with 5-FC in all cell lines, IC_{50} was lower. The results manifested that 5-FC can promote the oncolytic efficacy of oHSV-CD. In U87-Luc tumor model, bioluminescent imaging and MRI results demonstrated majority of injected tumors showed complete regression by treating with oHSV-CD or oHSV-CD together with 5-FC. Median survival was significantly increased compared with vehicle. Taken together, oHSV-CD/5-FC may be a promising therapeutic approach for glioblastoma treatment.

DRUG RESISTANCE

DRES-01. ZEB1-MEDIATED INVASIVE MESENCHYMAL TRANSITION AT THE SINGLE CELL LEVEL PROMOTES ANTI-ANGIOGENIC THERAPY RESISTANCE IN GLIOBLASTOMA

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INTRODUCTION: Bevacizumab responsiveness in glioblastoma is transient. The time course and upstream regulators of resistance still remain undefined. There is also conflicting evidence as to whether the resistance is driven by upregulated VEGF-independent angiogenic pathways or adaptation to treatment-induced hypoxia involving perivascular invasion. METHODS: We analyzed paired patient specimens before and after bevacizumab-resistance and two xenograft models of bevacizumab-resistance: (1) a multigenerational model that replicates the lengthy treatment duration in patients and (2) PDXs replicating patient tumor resistance. Transcriptional changes were studied using microarray and qPCR. Morphological changes were assessed by immunostaining; invasion was assessed by bioengineered 3D models of perivascular vs. parenchymal invasion. Stem cell enrichment was confirmed by stem cell reformation assays. RESULTS: Despite upregulated VEGF-independent pro-angiogenic genes, immunostaining revealed increased hypoxia and decreased vessel density in resistant xenografts and patient specimens, suggesting tumor growth despite effective bevacizumab-induced devascularization. Microarrays revealed overexpression of the mesenchymal subtype gene signature across resistant xenograft generations and in resistant PDXs, replicating patient specimens whose elevated mesenchymal gene signature correlated with bevacizumab treatment duration. Single-cell sequencing of bevacizumab-resistant patient specimens revealed these mesenchymal changes to arise in early cell clones with fewer mutations. Xenograft and patient specimen microarray analysis implicated ZEB1, a key mediator of mesenchymal transition and glioma-stemness, as a potential regulator of this change, with ZEB1 increasing across xenograft generations ($P< 0.001$). Late-generation resistant-xenografts revealed lower form factor ($p< 0.001$), increased perivascular and parenchymal invasion in 3D bioengineered models ($p< 0.001$ and $p< 0.05$), and larger neurospheres ($p= 0.002$) with higher stem cell counts ($p< 0.001$) versus to early-generations. CRISPR targeting of ZEB1 reversed the morphology, stem cell neurosphere formation, and mesenchymal gene expression changes defining resistance to that of bevacizumab-sensitive tumors. CONCLUSION: We identified ZEB1 as a targetable regulator of the mesenchymal change and associated perivascular invasion and stem cell enrichment defining bevacizumab resistance.

DRES-02. CILIARY PROTEIN ARL13B PROMOTES CHEMORESISTANCE BY MODULATE GLIOBLASTOMA PURINE BIOSYNTHESIS

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Glioblastoma (GBM) carries with it an almost 100% recurrence rate due to development of resistance to all conventional therapies. Our lab has demonstrated ARL13B, an ADP-ribosylation factor-like protein critical for cilia formation, plays an important role in promoting resistance to temozolomide (TMZ)-based chemotherapy. Knockdown of ARL13B in patient derived xenograft cells significantly increased survival of mice in an orthotropic GBM model when compared to controls ($p<0.0001$).The Cancer Genome Atlas (TCGA) dataset demonstrates time to recurrence in patients with downregulated ARL13B is substantially increased as compared to ARL13B upregulated patients (log-rank p-value=0.0012).To better understand the role of ARL13B in therapeutic adaptation we performed mass spectrometry analysis of an ARL13B pulldown during TMZ therapy and identified inosine monophosphate dehydrogenase 2 (IMPDH2), the rate-limiting enzyme in de-novo guanine nucleotide biosynthesis, as a significant binding partner of ARL13B during TMZ chemotherapy ($p<0.0001$). Immunoprecipitation analysis across multiple GBM cell lines validated this interaction and its increase during TMZ therapy. Probing this interaction further we examined the de novo and salvage purine biosynthesis pathways using radiolabeled carbon tracing experiments. In ARL13B knockdown cells, purine salvage pathway usage is upregulated 7-fold ($p<0.0001$) while de-novo pathway usage was decreased about 50% ($p=0.004$) in a TMZ specific manner. Moreover, ARL13B knockdown GBM cells treated with TMZ show a robust increase in DNA double-strand breaks compared to control cells exposed to TMZ, demonstrated by γH2X staining. Based on these observations, we hypothesize that ARL13B is a novel regulator of IMPDH2 allowing GBM cells to block salvage pathway biosynthesis to avoid TMZ induced DNA damage. However, when ARL13B is lost, GBM cells are forced into salvage pathway synthesis thus becoming sensitized to TMZ therapy due to increased incorporation of alkylated purines, a known function of TMZ.

DRES-03. EGFR-TARGETED THERAPY-INDUCED RESISTANCE MECHANISM IN MALIGNANT GLIOMAS

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Epidermal growth factor receptor (EGFR) is frequently amplified, mutated and overexpressed in malignant gliomas. Our investigation of the proximal and distal responses to EGFR inhibition identified the molecular mechanisms for the therapeutic resistance of EGFR inhibition in gliomas. Oncogenic EGFRviii-dependent gliomas initially showed cytotoxic responses upon removal of oncogenic cue. However, during the initial regression, a subset of tumor cells with activated mesenchymal subtype gene expression program emerged by lineage reprogramming which promoted tumor relapse in the absence of EGFRviii signaling. This lineage switch is dependent on YAP1 activation as a response to therapy, and is a key to EGFRviii independent tumor growth. YAP signature stratifies overall survival of recurrent glioma patients. Inhibition of YAP1 activation suppressed mesenchymal gene expression and significantly delayed recurrence of gliomas. Our findings provide mechanisms underlying inefficacies of EGFR targeted therapy in glioblastoma and suggest a new combinatorial targeting of EGFR and YAP for deeper and more durable responses.

DRES-04. CHARACTERIZATION OF A MODEL OF TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA

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Despite the discovery and widespread use of the chemotherapeutic drug temozolomide (TMZ), glioblastoma (GBM) remains a fatal cancer. TMZ, a DNA alkylating agent, provides a moderate survival benefit to patients whose tumours do not express the O6-methylguanine-methyltransferase (*MGMT*) gene. However, even these TMZ-sensitive GBMs recur and upon doing so, many are resistant to TMZ. The development of TMZ resistance is commonly associated with mutations in mismatch repair (MMR) and the re-expression of MGMT. Upregulation of homologous recombination (HR) and base excision repair (BER) have also been implicated as mechanisms of acquired TMZ resistance. To better characterize these mechanisms and to develop strategies to prevent or overcome resistance, our laboratory has implemented an *in vitro* model of inducible resistance in which frequent exposure to TMZ (100μM) yields multiple resistant colonies in the *MGMT*-methylated GBM cell line U251N (Yip et. al). These colonies displayed varying methods of resistance to TMZ, including those that have been clinically observed in recurrent, TMZ-treated GBMs. Several colonies harboured mutations in MMR genes *MSH6, MSH2*, and *MLH1* with low or absent expression of their respective proteins. In addition, some MMR wild-type colonies had increased expression of poly-ADP ribose (a polymer required for the recognition of DNA breaks by the BER machinery), suggesting that upregulation of BER may be driving resistance. Furthermore, Western Blot analysis revealed that occasional colonies re-expressed MGMT. Interestingly, a few colonies did not possess these alterations, suggesting that their resistance may result from further downstream modifications of MMR or BER, or by mutations in HR. With a more comprehensive characterization of these U251 colonies, we hope to learn more about TMZ resistance in GBM, and refine treatments or preventative therapies for molecularly distinct, TMZ-resistant, recurrent tumours.

DRES-05. MOLECULAR EVOLUTION OF DIFFUSE GLIOMAS AND THE GLIOMA LONGITUDINAL ANALYSIS CONSORTIUM

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A comprehensive characterization of the somatic alterations and molecular subtypes of glioma at diagnosis has been established. However, gliomas undergo significant molecular changes over time, some of these causing malignant progression or associated with therapy. Understanding this molecular evolution may uncover therapeutic vulnerabilities and facilitate development of more effective systemic therapies. The Glioma Longitudinal Analysis (GLASS) Consortium is an international effort to systemically catalogue the longitudinal changes in gliomas through multi-platform characterization. GLASS has developed computational and clinical infrastructure to enable its mission of establishing a well annotated longitudinal molecular dataset of 1500 gliomas including 500 gliomas per each of the IDHwt/IDH-mut-non-codel/IDH-mut-codel subtypes. An initial dataset comprising 150 cases with exome sequencing at multiple timepoints has been constructed, including cases diagnosed as IDHwt (55%), IDH-mut-non-codel (38%) and IDH-mut-codel (6%). Treatment regimens followed the expected combinations of chemo- and radiation therapy while survival was more favorable than reported in literature, suggested a bias in our dataset towards better performing cases. This is possibly explained by the need for two consecutive surgical procedures to be included in the analysis. While more than half of mutations in IDHwt primary tumors were recovered in their matching recurrences, this fraction was less than 30% in the other two subtypes. The disparity in mutations between primary and recurrences may reflect intratumoral heterogeneity in both primary and recurrence, and clonal selection patterns. We observed temozolomide treatment-associated hypermutation in up to 13 of our cohort. A more detailed analysis of mutational and DNA copy number data is underway. A cohort of 75 additional patients with multi-timepoint exome data is being processed and, through several funded projects, multi-platform characterization of 250 gliomas is in progress. In summary, GLASS will provide a rich resource to the glioma community with the potential for paradigm shifting discoveries.

DRES-06. PREVENTING THE EMERGENCE OF TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA BY PARP-1 INHIBITION

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The DNA alkylating agent temozolomide (TMZ) is active against the subset of glioblastomas (GBMs) in which the O6-methylguanine DNA methyltransferase (*MGMT*) gene is silenced by promoter methylation. However, despite their initial sensitivity, virtually all *MGMT*-methylated tumors acquire drug resistance and regrow in patients. Previously, we tested the hypothesis that inhibiting poly(ADP-ribose) polymerase-1 (PARP-1) might increase the potency of TMZ in resistant GBMs. Our results showed that primary cell lines derived from TMZ-resistant GBMs were re-sensitized by pre-treatment with the PARP-1 inhibitor ABT-888. While PARP-1 inhibitors may be useful for treating resistant cases, we predict that greater clinical benefit can be derived from its use in sensitive cases where inhibition may prevent the emergence of resistant clones. To test this hypothesis, we established an *in vitro* model of inducible resistance in the *MGMT*-methylated, TMZ-sensitive U251N GBM cell line. In this model, prolonged treatment of the U251N line with TMZ resulted in the emergence of resistant colonies of cells with *de novo* alterations in DNA repair pathways. Of note, sanger sequencing and western blot analyses revealed that some resistant colonies harbored mutations in mismatch repair (MMR) genes *MSH2*, *MSH6* and *MLH1* and have reduced expression of MSH2 and MSH6. These observations are strikingly similar to the recurrent, TMZ-refractory human disease where mutations in and downregulated expression of MMR genes are frequently reported. In another set of experiments, co-treatment of the parental U251N line with TMZ and ABT-888 prevents resistant colonies from emerging. Moreover, co-treatment causes established resistant colonies to regress, suggesting that PARP-1 inhibition can target and prune the evolution of TMZ-resistant populations. In summary, this work has the potential to show that PARP-1 inhibition may prevent or delay disease progression and prolong the life of patients who inherently benefit from TMZ therapy.

DRES-07. DEFINING THE MECHANISMS OF ACQUIRED RESISTANCE TO TYROSINE KINASE INHIBITORS IN EGFR-DRIVEN GLIOBLASTOMAS USING INTEGRATED KINOME AND TRANSCRIPTOME PROFILING

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Glioblastoma (GBM), the most common and malignant adult primary brain tumor, has been extensively molecularly characterized. Two mutations occur at particularly high frequency: *CDKN2A* deletion (50–60%) and *EGFR* (40–50%), especially EGFRvIII (~35%) in which exons 2–7 are deleted and result in constitutive kinase activation. EGFR is the most attractive therapeutic target due to frequent activating mutations and ready availability of multiple targeted inhibitors. Several EGFR-targeted tyrosine kinase inhibitors (TKI) have failed clinically, due in part to intrinsic and acquired resistance. To examine these mechanisms, we are using genetically-engineered mouse astrocytes harboring homozygous deletions of *Cdkn2a*, as well as the activating EGFRvIII mutation (CEv3). To model acquired resistance, CEv3 astrocytes were made intrinsically resistant to the EGFR TKI gefitinib or erlotinib via long-term exposure, both *in vitro* and *in vivo*. We found that long-term gefitinib or erlotinib exposure confers variable levels of cross resistance to a panel of second- and third-generation EGFR-TKI (ΔIC_{50} 1.12-36.1-fold), relative to non-resistant parent lines. We have previously shown that dynamic kinome reprogramming may be responsible for TKI resistance. Therefore, we are using a chemical proteomics method, multiplexed inhibitor beads and mass spectrometry (MIB-MS), to examine changes in the expressed and functional kinome, in both the presence or absence of one of several EGFR-TKI known to penetrate the blood-brain barrier. Additionally, we are performing RNA sequencing (RNA-seq) to inspect transcriptomic alterations in response to these drugs. Preliminary RNA-seq results showed that resistant CEv3 mouse astrocytes clustered separately from their non-resistant *in vitro* and *in vivo* counterparts. Together, data from these experiments will create a framework of transcriptomic and proteomic changes that occur in murine models of GBM with defined mutational profiles. This framework can then be used to help define novel therapeutic targets that could significantly alter the current treatment paradigm of GBM.

DRES-08. DYNAMIC KINOME PROFILING OF GENETICALLY-DEFINED, EGFRvIII-DRIVEN MURINE ASTROCYTE MODELS OF GLIOBLASTOMA REVEALS TARGETS FOR DUAL KINASE INHIBITOR THERAPY

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Glioblastoma (GBM) has poor survival and lacks effective treatments. Due to frequent amplification and mutation of epidermal growth factor receptor (EGFR), several EGFR tyrosine kinase inhibitors have been trialed, but none have proven successful. One potential reason for failure is acquired resistance, particularly acute, adaptive responses in the kinome. To study this adaptive resistance mechanism, we used RNA-seq and multiplex inhibitor bead/mass spectrometry (MIB-MS) to analyze transcriptomes and kinomes of genetically-engineered murine astrocytes with genotypes commonly seen in human GBM. We previously showed that 38% (86 of 228) of the expressed kinome varied among a panel of genetically diverse murine astrocytes harboring *Cdkn2a* deletion (C) plus *Pten* deletion (CP), wild-type human EGFR (CE) or EGFRvIII (CEv3) overexpression, or both overexpressed EGFRvIII and *Pten* deletion (CEv3P). Pairwise genotype comparisons revealed multiple differentially activated kinases, including *Pdgfrb*, *Fgfr2*, *Lyn*, *Ddr1*, and several members of the Ephrin family. We further investigated these potential targets for dual therapy with EGFR TKI by examining the transcriptional response of our cultured astrocyte panel at 4, 24, and 48 hours after 3 μM afatinib. Afatinib induced no kinome changes in C and only 3 kinases (*Fn3k*, *Prkg2*, and *Syk*) were altered in CP astrocytes. Despite similar baseline gene expression profiles, CE astrocytes overexpressing wild-type EGFR responded significantly differently than C astrocytes without. Five kinases (*Dclk1*, *Epha3*, *Epha7*, *Fgfr3*, and *Prkg1*) were induced, while 14 were repressed. Six were similarly repressed in CEv3 (*Bub1*, *Nek2*, *Pask*, *Plk4*, *Prkcb*, and *Vrk1*). Whereas the kinase transcriptome response was blunted in C, CP, and CE astrocytes, afatinib induced altered expression of significantly more kinases in CEv3 (82) and CEv3P cells (49). One particularly attractive target in CEv3 astrocytes was *Epha4*, which afatinib induced >40-fold. Dual inhibition of EGFRvIII and Epha4 kinases may thus provide an opportunity for more effective targeted therapy.

DRES-09. IN VIVO FUNCTIONAL GENOMICS IDENTIFIES DRIVERS OF CHEMORESISTANCE IN MEDULLOBLASTOMA

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Brain tumours are the main cause of cancer-related death during childhood and medulloblastoma an aggressive embryonal tumour that arises in the posterior fossa – is the most common malignant tumour in this age group. Chemotherapy is a cornerstone of the postsurgical treatment, particularly in younger children in whom craniospinal irradiation is omitted due to the devastating side effects in the developing brain. Medulloblastoma often progresses or recurs after chemotherapy with a dismal prognosis. We used the Sleeping Beauty (SB) transposon-driven Ptch+/–/Math1-SB11/T2Onc sonic hedgehog (SHH) medulloblastoma murine model as a functional genomic tool to perform a genome-wide screen and identify genes and pathways that promote resistance to chemotherapy. After sub-total resection of the primary tumours, the mice were treated with repeated cycles of chemotherapy (cisplatin 5 mg/kg IP once on day 1 followed by cyclophosphamide 150 mg/kg IP daily from day 2 – 5) every 2 weeks for up to 3 cycles and monitored for tumour recurrence. The primary tumours (pre-treatment) and the tumours and metastasis that regrew after chemotherapy were deep sequenced to determine the transposon insertion sites. We identified recurrence-specific clonally selected insertions that promoted tumour growth despite therapy, including p53 (recurrently mutated in human tumours at relapse) and several other genes involved in DNA repair. Using cerebellar orthotopic models of p53-mutated SHH medulloblastoma, we observed a significant improvement in survival when the ATM inhibitor AZ32 was added to the chemotherapy backbone. This provides a rationale for developing therapeutic approaches targeting DNA repair in combination with conventional chemotherapy to prevent chemoresistance and medulloblastoma recurrence.

DRES-10. DRD5 IS A MODULATOR OF GLIOMA SUSCEPTIBILITY TO DRD2 ANTAGONISM BY ONC201

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ONC201 is the first selective antagonist of dopamine receptor D2 (DRD2) and D3 (DRD3) for clinical oncology that has exhibited preliminary clinical activity in high grade gliomas. We investigated DRD2 dysregulation in glioma and its role in ONC201 efficacy. Investigating CRISPR screens across a spectrum of cancer revealed that glioma cell lines had the highest DRD2 gene essentiality scores, indicating that glioma is a tumor type with the most vulnerability to DRD2 antagonism. Investigation of TCGA revealed that DRD2 is highly expressed in glioblastoma relative to other dopamine receptor family members and is associated with a relatively poor clinical prognosis. Tissue microarray analysis confirmed DRD2 overexpression in glioblastoma relative to normal brain. A linear correlation between DRD2 mRNA and ONC201 GI50 was observed among NCI60 glioblastoma cell lines. Similarly, we found a significant concordance between a cell line's sensitivity to ONC201 within the Genomics of Drug Sensitivity in Cancer (GDSC) panel and its DRD2 gene essentiality score. Next, we ranked the relative contribution of each dopamine receptor to ONC201 efficacy using a bioinformatics approach based on a generalized linear model. We found that the strongest negative contributor was DRD2 – where a negative contribution denotes a decreased IC50 value as expression increases. Interestingly, DRD5, a D1-like dopamine receptor that counteracts DRD2 signaling, was measured as having the highest positive score – indicating that low expression of DRD5 was correlated with ONC201 efficacy. DRD5 expression was significantly inversely correlated with ONC201 potency in the NCI60 and GDSC datasets. Furthermore, a missense DRD5 mutation was identified in tumor cells with acquired resistance to ONC201. Resistance could be recapitulated with overexpression of the mutant or wild-type DRD5 gene. In conclusion, DRD2 dysregulation and DRD5 expression predict preclinical ONC201 glioma sensitivity that may be used to identify additional settings for clinical evaluation.

DRES-11. A SYSTEMS APPROACH FOR DETERMINING THE MECHANISM OF RESISTANCE TO TUMOR TREATING FIELDS IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and deadliest malignant brain cancer in adults despite aggressive chemoradiotherapy. Recently, Tumor Treating Fields (TTFields) were approved in combination with adjuvant temozolomide chemotherapy for newly diagnosed GBM. The addition of TTFields resulted in a significant improvement in overall survival. TTFields are low-intensity alternating electric fields that are thought to disturb mitotic macromolecules' assembly, leading to disrupted chromosomal segregation and cell death. However, many TTFields responders eventually develop progression. The mechanism of TTFields resistance remains largely unexplored. Understanding how cancer cells gain the ability to circumvent the biophysical forces of TTFields and their downstream effects will provide new opportunities to improve therapeutic efficacy of this novel anti-cancer treatment. To accomplish these objectives, we have developed several human GBM cell lines that demonstrated relative resistance to the cytotoxic effects of TTFields compared to the parental cells. Importantly TTFields-induced chromosomal instability such as the formation of micronuclei was unchanged in resistant cells compared to their sensitive counterparts. In contrast, TTFields-induced inflammatory response was severely suppressed in resistant cells, suggesting that resistance to TTFields may be conferred by a selective loss of the deleterious effects downstream of the biophysical insults. Importantly, this acquired TTFields resistance phenotype of GBM cells was associated with a transition to a stem-like state. Using a systems approach aided by a suite of innovative computational platforms, we methodically dissected this renewed stemness program in resistant cells to identify master regulators of the resistance mechanism. Our long-term goal is to develop targeted therapies that prevent tumor's resistance to TTFields.

DRES-12. FUNCTIONAL GENETIC APPROACHES TO OVERCOME TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA

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Glioblastoma (GBM), the highest grade of malignant astrocytomas, is the most common and lethal primary central nervous system tumour in the adults. Despite the recent advances in treatment modalities, GBM patients generally respond poorly to all therapeutic approaches and prognosis remain dismal. Radiation and chemo-resistance are characteristic of various cancer types, however it is not clear if this therapy resistance is a consequence of tumour progression or it is intrinsically associated with the genetic events that lead to the tumour formation in the first place. Gaining insights into the pathways that determine this poor treatment response will be instrumental for the development of new therapeutic modalities. Alterations of the DNA damage response (DDR) have been associated with therapy resistance, offering both challenges and opportunities from a treatment prospective. Currently, a number of laboratories are exploring the possibility of manipulating the DDR to cause selective tumour cell death through mitotic catastrophe. In order to identify genes that modulate temozolomide (TMZ) response we have performed a series of in vitro shRNA screenings, using a customized sRNA library against DDR genes and several GBM cell lines with various genetic makeup (e.g.: MGMT+, MGMT-, MMR-proficient and MMR-deficient). Our studies allowed to pinpoint both positive and negative regulators of TMZ sensitivity. Novel approaches to overcome TMZ resistance will be presented.

DRES-13. VEGF BLOCKADE ENHANCES T REGULATORY CELL FUNCTION BY DYSREGULATING GLUTAMATE TRANSPORT IN GBM

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In treating patients with cancer, inhibition of the VEGF pathway has been shown to prolong recurrence-free survival (RFS). However, anti-VEGF therapy has failed to improve overall survival in patients in randomized clinical trials. To help patients who are undergoing anti-angiogenic treatment, we attempted to tackle the obstacles of the treatment, using glioma animal models. In contrast to most literature reports focusing on the immediate post-treatment effect, we mainly investigated the animals that had failed the treatment. We found that tumor immunosuppression plays a critical role in treatment failure. Higher-doses of anti-VEGF treatment resulted in an over-representation of Tregs in tumor-infiltrating lymphocytes (TILs) and elevated expression of checkpoint molecules on TILs. The therapy mediates overexpression of xCT (a glutamate/cystine antiporter), which leads to excessive glutamate (a neurotoxin) production by glioma cells. The glutamate is responsible for the enhanced Treg suppressive function, which is signaling through upregulation of glutamate receptor 1(mGluR1) on the Tregs. Depleting CD25+ T cells prior to anti-VEGF therapy restores T cell production of IFN-g, enhancing the antitumor response. Collectively, VEGF blockade exacerbates Treg suppressive function by dysregulating glutamate antiport, and co-administration of Treg depletion with VEGF blockade can provide a synergetic antitumor effect for treating GBM.

DRES-14. PROTEIN AGGREGATE FORMATION PREDICTS CLINICAL RESPONSES TO EGFR TKIs

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BACKGROUND: EGFR tyrosine kinase inhibitors (TKIs) have thus-far not shown clinical benefit for glioma patients, which is in stark contrast to the benefit observed for patients with EGFR-mutated pulmonary adenocarcinomas (PA). We performed functional analysis on various EGFR-mutation constructs to better understand the differential sensitivity to EGFR TKIs. METHODS: Response to EGFR TKIs was monitored using high-throughput automated imaging analysis, western blot, immunofluorescence, RT-QPCR, and immunoprecipitation-mass spectrometry (IP-MS). RESULTS: Addition of TKIs to cells ectopically expressing EGFR mutation constructs resulted in a rapid and strong formation of EGFR-protein aggregates. However, aggregates were found only cells expressing mutations common to PAs (n=3 mutations tested) but not in mutations common to gliomas (n=4) or those harboring secondary resistance mutations (n=1). Moreover, aggregates were only observed in TKIs with proven clinical benefit (erlotinib, gefitinib, dacomitinib or osimertinib) and not with a drug with no such benefit (lapatinib). We find a high concordance between the IC50 for viability of 13 cell lines harboring endogenous EGFR mutations (n=12 different mutations) and the IC50 of mutations to form protein aggregates. Patients harboring mutations that are sensitive to aggregate formation (IC50 < 500 nM) had significantly longer time to progression (median survival 7.5 vs 13 months, HR 0.25, P=0.012). These data demonstrate that formation of aggregates predicts response to EGFR TKIs in patients. The aggregates are formed, predominantly in the nucleus, first by a dephosphorylation of EGFR after which specific mutations and drugs both affect the (inactive) conformation state of EGFR. Such a state renders the protein more prone to aggregate formation and subsequent complete inactivation. CONCLUSION: Protein aggregation predicts response to EGFR TKIs. Since these aggregates inactivate all functions of the protein, the absence of aggregate formation with glioma specific mutations in EGFR explain why these tumors are insensitive to EGFR TKIs.

DRES-15. GSK3 BETA AND hnRNPA1 (RNA BINDING PROTEIN) COOPERATES WITH cMyc TRANSCRIPTIONAL REGULATOR, ANTAGONIZES GSK3 ALPHA UNDER THERAPEUTIC STRESS

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Oncogenic cooperation activity tends these glioma cells to acquire drug resistance. Where GSK3 beta isoform cooperates cMyc antagonizes GSK3 alpha isoform. Down regulated expression of GSK3 alpha cooperates elevated hnRNPA1 expression, evolving glioma development. Occurrence of hnRNPA1 dependent alternative oncogenic variant is one of the resistance mechanism. Inhibiting GSK3 beta isoform using specific siRNAs is one the approach rather than to inhibit GSK3 kinase activity, also downregulate hnRNPA1 which is cMyc dependent. Using Chromatin Immuniprecipitation approach we observed potential differences in between transcriprional regulators of GSK3 beta and GSK3 alpha isoform. Positive feedback mechanism of alpha and beta isorform of GSK3 to stabilize cMyc, therefore inhibiting expression of heterogenous ribonucleor protein family members. Interfering the positive feed back GSK3 beta- cMyc-GSK3 beta, is major hallmark to catch on cMyc transcriptional regulator. In another experiment inhibition of PI3 kinase/ AKT pathway using specific inhibitors elevates GSK3 beta and hnRNPA1 but not GSK3 alpha, associates with cMyc, indicating potential role of GSK3 beta isoform in glioma resistance development. Immunohistochemistry based analysis shows inverse correlation between GSK3 alpha and hnRNPA1.

DRES-16. HYPOXIC MICROENVIRONMENT DETECTED BY FRP-170 PET MAY INDUCE THE EXPRESSION OF DRUG RESISTANCE GENES IN THE PATIENT WITH GLIOBLASTOMA

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The brain tumor cells can survive regardless of hypoxia and undernutrition. Hypoxia is known to be causes of resistance to radio-chemotherapy, increased recurrent and poor prognosis. Recent reports demonstrated that cancer stem cells (CSCs) exist in hypoxic lesion of the tumor. In this study, we examined the mechanism of drug resistance under hypoxic condition *in vitro* and *in vivo*. Most of brain tumor patients are treated by temozolomide (TMZ) as chemotherapy. The efficiency and resistance of TMZ are associated with the O^6-methylguanine-DNA methyltransferase (MGMT) gene. In addition, MGMT expression is induced by hypoxia, and regulated by N-myc downstream regulated gene 1 (NDRG1). We investigated the expression of these genes in glioma cell lines (GCs), glioma stem cells (GSCs), and clinical specimens detected by molecular hypoxia imaging, 18F-FRP170 PET. Some hypoxic clinical specimens showed the increased expression of MGMT and NDGR1 genes. However, GBM cell lines and GSCs showed increased expression of NDRG1 and decreased expression of MGMT. These data suggest that a hypoxic microenvironment in vivo might play an important role in molecular and phenotypic profile of tumor cells including cancer stem cells.

DRES-17. ACTIVATION OF FGF SIGNALING PATHWAY CONFERS RESISTANCE TO EGFR INHIBITION IN GBM

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Amplification and mutation of the EGFR gene is a signature lesion in GBM and occurs in 40–50% of GBM patients. However, EGFR inhibition has been ineffective in the treatment of GBM. Resistance to EGFR inhibition may be primary or secondary after an initial period of susceptibility suggesting that adaptive mechanisms may mediate resistance to EGFR inhibition The adaptive response to EGFR inhibition is multifaceted. We have recently reported that TNF-JNK-Axl-ERK signaling axis mediates primary resistance to EGFR inhibition in GBM cells[1]. We now report that the FGF (fibroblast growth factor) signaling pathway is activated by EGFR inhibition in GBM. A number of studies have identified the FGF signaling pathways as important pro-oncogenic signals in cancer and in mediating resistance to treatment. Our data indicate that the FGFR pathway is an additional candidate for mediating the adaptive response to EGFR inhibition. We find that in glioma cells expressing either EGFRwt or the mutant EGFRvIII, EGFR inhibition with erlotinib induces FGF1 and FGF3 expression at both mRNA and protein levels. Additionally, erlotinib exposure leads to phosphorylation of FGFR1 in multiple GBM cell lines. Knockdown of FGFR1 sensitized GBM cells to EGFR inhibition. Moreover, combined treatment of erlotinib and FGFR selective inhibitors such as Debio-1347 or AZD4547 significantly increased cell death versus in either drug alone in multiple GBM cell lines. Our findings suggest that combined EGFR and FGF inhibition could be an effective alternative approach to treating GBM. 1. Guo G, et al. A TNF-JNK-Axl-ERK signaling axis mediates primary resistance to EGFR inhibition in glioblastoma. Nat Neurosci. 2017;20(8):1074–1084.

DRES-18. SUMO1 AND VALOSIN-CONTAINING PROTEIN REGULATE RETINOID RECEPTOR PROTEIN TURNOVER– A PROCESS DISRUPTED IN GLIOBLASTOMA

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BACKGROUND: Resistance to therapeutic use of retinoids in glioblastoma (GBM) has been observed for over 20 years; however, the exact mechanism of resistance remains unknown. To better understand retinoic acid resistance in GBM, we studied the turnover mechanism of retinoid receptor proteins in normal neural stem cells and glioma stem-like cells (GSCs). Currently, it is believed that cellular stress induces global sumoylation of proteins in glioma, yet the precise role of sumoylation is not fully understood. METHODS: Protein expression and posttranslational modification (PTM) of the endogenous retinoid receptors were analyzed using Western blots, immunoprecipitations, and siRNA. The discovery of the novel binding partner of retinoid receptors was achieved using immunoprecipitation and mass spectrometry. Promoter luciferase assays were used to measure transcriptional activities. RESULTS AND CONCLUSIONS: Our studies reveal that sumoylation of retinoid receptors occurs in both normal neural stem cells and GSCs; however, protein turnover of the receptor is disrupted in glioma. We show that sumoylation is a PTM required for proteasomal degradation of retinoid receptors. Degradation via the proteasomal pathway is necessary for receptor protein turnover and transcriptional activity. We also identify that the valosin-containing protein (VCP/p97/Cdc48) participates in the PTM of retinoid receptors and impacts the transcriptional activity. The defect in glioma occurs after the sumo modification step and results in the accumulation of high molecular weight forms of the receptors that fail to get degraded. Our findings expand our understanding of the turnover mechanism of nuclear receptors in normal cells. In addition, our findings provide a mechanism for the retinoic acid resistance in glioma cells that involves the disruption of protein turnover and decrease in transcriptional activity. Our studies suggest that the use of combinatory therapies that target retinoid receptors and induce proteasomal degradation of the receptors to ensure protein turnover may be a more effective approach.

DRES-19. THE MECHANISMS OF RESISTANCE TO TEMOZOLOMIDE IN GLIOMA CELLS

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Glioblastoma is one of the most aggressive tumors in the central nervous system tumors, with 5-year survival rates of less than 10%. The standard therapy for glioblastomas is maximal safe resection, followed by radiation therapy and chemotherapy with temozolomide (TMZ). One of the reasons of the worse prognosis is the acquisition of resistance to TMZ. TMZ is a DNA-methylating agent, delivering a methyl group to DNA (O6-guanine, N7-guanine and N3-adenine). The primary cytotoxic lesion, O6-methylguanine, mispairs with thymine, leading to futile DNA mismatch repair (MMR), formation of double strand breaks and eventual cell death, in the absence of O6-methylguanine DNA methyltransferase (MGMT). To clarify the mechanisms of resistance to TMZ and to find the way to overcome the resistance to TMZ, several clones of TMZ-resistant U251 were obtained and analyzed. #3 clone showed G2 arrest after TMZ exposure and this arrest was abrogated sooner compared to parental U251. TMZ did not induce G2 arrest in #8 clone. The expression of MGMT was not found in U251 parental cells, #3 cells nor #8 cells. The ability of homologous recombination (HR) was increased in #3 clone, and by suppression of HR, #3 resistant clone was resensitized to TMZ, however #8 was not. The protein levels of MSH6, which was associated with MMR, was reduced in #8 clone. PARP inhibitor resensitized #8 clone to TMZ, inducing apoptosis. Inhibition of HR or base excision repair was suggested to be a useful strategy to resensitize TMZ-resistant gliomas with higher HR or with MMR dysfunction to TMZ, respectively.

DRES-20. THE TNF RECEPTOR FAMILY MEMBER Fn14 IS HIGHLY EXPRESSED IN RECURRENT GLIOBLASTOMA (GBM) AND IN GBM PATIENT-DERIVED XENOGRAFTS WITH ACQUIRED TEMOZOLOMIDE RESISTANCE

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INTRODUCTION: The current standard-of-care for patients with glioblastoma (GBM) consists of maximal safe surgical resection followed by high-dose radiation and concomitant oral chemotherapy using the DNA-alkylating agent temozolomide (TMZ). Despite this combination treatment, tumor recurrence is nearly universal. Recurrent GBM tumors tend to be more aggressive and treatment-resistant than their primary (i.e. newly diagnosed) counterparts. A better understanding of recurrent GBM biology would offer new opportunities for tailoring treatments following standard-of-care chemo-radiation therapy. Our prior work has demonstrated an important role for the TWEAK receptor Fn14 in GBM patho-biology. In the current study, we investigated Fn14 expression in recurrent GBM and in the setting of TMZ resistance. METHODS: Fn14 mRNA expression levels in non-neoplastic brain, primary GBM, and recurrent GBM (post-chemotherapy and radiation) specimens were obtained from the TCGA data portal. Immunohistochemistry was performed using non-neoplastic brain as well as patient-matched primary and recurrent GBM specimens to examine Fn14 protein levels. Western blot analysis was used to compare Fn14 expression in parental TMZ-sensitive or matched TMZ-resistant patient-derived xenografts (PDXs) established from primary or recurrent tumor samples. The migratory capacity of control and Fn14-depleted TMZ-resistant GBM cells was assessed using the transwell migration assay. RESULTS: We found that Fn14 is more highly expressed in recurrent GBM tumors than their matched primary GBM counterparts. GBM PDX cells with acquired TMZ resistance have higher Fn14 levels and greater migration capacity than their corresponding parental TMZ-sensitive cells, and the migratory difference is due, at least in part, to Fn14 expression in the TMZ-resistant cells. CONCLUSIONS: This study demonstrates that the Fn14 gene is highly expressed in recurrent GBM and TMZ-resistant GBM PDX tumors. These findings suggest that Fn14 may be a valuable therapeutic target or drug delivery portal for treatment of recurrent GBM patients.

EPIDEMIOLOGY

EPID-01. ASSOCIATIONS OF TIMING OF ADJUVANT THERAPIES, RADIATION FRACTIONS AND RADIATION DOSES WITH GLIOBLASTOMA SURVIVAL: A RETROSPECTIVE COHORT ANALYSIS USING THE NATIONAL CANCER DATABASE AND SEER-MEDICARE DATABASE

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Few studies investigated the association between the timing of initiating adjuvant therapies and survival in glioblastoma (GBM) patients. A total of 20511 and 4435 eligible GBM patients were derived from the National Cancer Database (NCDB) and the Surveillance, Epidemiology and End Results (SEER) - Medicare dataset, respectively (NCDB: 2005–2014; SEER-Medicare: 2004–2013). Times to starting adjuvant treatment were calculated as the days from the date of diagnosis to the initiation of adjuvant treatment [radiation therapy (RT), chemotherapy, or concurrent chemoradiation (CRT)] and categorized into quartiles (Q1: 0–21; Q2: 22–30; Q3: 31–39; Q4: ≥40, days). Kaplan-Meier method and Cox proportional hazards regression were applied for survival analysis. Multivariate logistic regression was performed to compare differences in treatment patterns, delayed treatment, and secondary outcomes. The patients underwent biopsy obtained significant survival benefit by having adjuvant treatment during Q2 and Q3 [NCDB: HR: Q1 (Ref.), Q2: 0.88, Q3: 0.86, Q4: 0.91; SEER-Medicare: Q1 (Ref.), Q2: 0.87, Q3: 0.86, Q4: 0.89]. For the patients with craniotomy, initiation of adjuvant treatment during Q2 and Q3 had significantly reduced risk of death [NCDB: HR: Q1 (Ref.), Q2: 0.95, Q3: 0.94, Q4: 1.03; SEER-Medicare: Q1 (Ref.), Q2: 0.98, Q3: 0.96, Q4: 1.00]. Furthermore, patients received more RT fractions [comparing to 10–29 fractions, 30–33 fractions: HR: 0.62 (biopsy), 0.62 (resection); ≥34 fractions: HR: 0.53 (biopsy), 0.62 (resection)] and higher-dose RT [comparing to 34–46 Gy, 50–60 Gy: HR: 0.91 (biopsy), 0.95 (resection); ≥ 60 Gy: HR: 0.77 (biopsy), 0.88 (resection)] experienced significantly survival benefit in both biopsy and resection groups. A similar analysis was performed in SEER-Medicare dataset as validation set and the findings remained consistent. The impact of time to adjuvant treatment on GBM survival varied by surgery procedures. Having adjuvant treatment immediately may not guarantee a significant survival benefit. More RT fractions and higher-dose RT are associated with better survival.

EPID-02. EFFECTS OF TREATMENT AND SOCIAL DEMOGRAPHICS ON ADULT MEDULLOBLASTOMA SURVIVAL

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BACKGROUND: Medulloblastoma in adults is exceedingly rare, and treatment strategies are largely derived from data in the pediatric medulloblastoma population. The aim of this study was to evaluate treatment patterns and survival in adults with medulloblastoma. We performed a retrospective study of the California Cancer Registry (CCR), to evaluate the effect of treatment variables and social demographics on survival in adults. METHODS: Treatment and demographic data were extracted from the CCR for adult patients (18 years and older) with medulloblastoma. Patients were diagnosed from January 1, 1988 through December 31, 2010. We restricted the data to patients who had complete records for extent of resection, radiation, chemotherapy and demographic variables. Surgery was dichotomized to Gross Total Resection (GTR) and Subtotal Resection/Biopsy (STR/B). A total of 292 patients were included. Kaplan Meier (KM) survival curves were performed for OS. Bivariate and multivariate analyses were done by cox proportional hazard regression model for all variables. RESULTS: There were 245 cases of classic adult medulloblastoma, 40 desmoplastic, 6 large cell and 1 medullomyoblastoma. 37% underwent GTR and 63% had STR/B. 82% received radiation and 42% of the patients underwent some form of chemotherapy (single agent or multiagent). Median OS for patients undergoing GTR was not reached, vs 115 months for STR/biopsy (HR 0.585). Median OS for patients with radiation was significantly associated with improved OS (211 months vs 56 months, (HR 0.450). There was no survival advantage for patients who received chemotherapy (155 months with chemotherapy vs 180 months without, (HR 1.081). Multivariate analysis again showed GTR and radiation to be significant predictors of OS, but not chemotherapy. Marital status and age were significant predictors of OS, but not race or gender. CONCLUSION: Radiation and GTR were significantly associated with longer OS. Chemotherapy was not associated with an improvement in OS.

EPID-03. THE MINORITY ADVANTAGE -DISPARITIES IN PRIMARY BRAIN TUMOR SURVIVAL IN TEXAS STATE 1995–2013

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BACKGROUND: This paper examines demographic and geographic disparities in primary brain tumor survival in Texas state, predictors of survival and hazard ratios and will reflect differences at the local level that would have otherwise been masked in a national report. IMPACT: Our study reveals evidence of genetic and environmental basis in primary brain tumor survival. Insights into specific disparities can guide research especially on poorly understood entities, health policy implementation and local practice. METHODS: Data was obtained from the Texas cancer registry 1995–2013.event was death from malignant primary brain tumor and cause specific survival was used. SAS version 9.3 and SEER*Stat 8.3.2 were used for the analysis. Kaplan Meier survival estimates with the Log-Rank test was used to compare the survival rates. The Cox regression proportional hazard model estimated the hazard ratios. RESULTS: There is significant demographic difference in survival curves. White males were at greatest hazard of dying from malignant brain tumors. Median survival is least among Non-Hispanic whites: 20 months (95% CI: 19, 21) and greatest among Asian: 92 months (95% CI: 72, 142). 5 year survival was White(38.9%), Blacks (44.3%) American Indian/Alaskan native (55.9%) Asian (56.7%). There is observed inverse relationship between socio-economic status and survival. Other significant predictors of survival were age, sex and geographic location. CONCLUSION: Ethnic minorities and lower socio-economic class demonstrated survival advantage. Non- Hispanic white males have worse survival of primary brain tumors. Survival curves differed significantly by sex, geographic location and race. Race, age, sex, geographic location and poverty level significantly predict the hazard of death reflecting strong genetic and environmental influence.

EPID-04. LEVERAGING GENOMIC DATA TO IDENTIFY RISK FACTORS FOR CHILDHOOD EPENDYMOMA

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SNPs identified by previous GWAS can serve as instrumental variables for exposures of interest using a process known as Mendelian randomization. We sought to identify risk factors for childhood ependymoma using a Mendelian randomization approach. We identified 390 Californian children born after 1982 and diagnosed with intracranial ependymoma before age 19, and retrieved their archived newborn bloodspots from the state. DNA from 360 cases and 2176 controls was successfully extracted and genotyped on the Affymetrix Axiom array which, coupled with whole-genome imputation, yielded 4.5M SNP genotypes per subject. We constructed polygenic scores of anthropometric traits, telomere length, and immune cell profiles using SNPs discovered in prior GWAS. Case-control comparisons were adjusted for ancestry-informative principal components. A polygenic score for adult height (416 unlinked SNPs) indicated that a 2.5cm increase in predicted height was associated with a 1.23-fold increased risk of ependymoma (P=0.041). No associations were observed for birth length, pre-pubertal height, or head circumference. A polygenic score for inter-individual variation in telomere length (8 unlinked SNPs) indicated that longer predicted telomere length increases risk of ependymoma (P=0.037), particularly adolescent-onset ependymoma ($P=7.1x10^{-3}$). A polygenic score for lymphocyte count (156 unlinked SNPs) was not associated with ependymoma risk (P=0.14). However, a one standard deviation increase in the score for myeloid cell count (191 unlinked SNPs) was associated with a 0.79-fold decreased risk of ependymoma ($P=9.0x10^{-3}$). Within the granulocyte lineage, increased neutrophil count was associated with decreased risk of ependymoma (OR=0.82, P=0.027), while neither eosinophil count nor basophil count showed evidence of association (P=0.22 and 0.99, respectively). Because ependymoma therapy can affect height attainment, telomere length, and immune parameters, these exposures are ill-suited for traditional case-control study designs. Using a Mendelian randomization approach, we observe that genetic predisposition to taller height, longer telomere length, and decreased circulating myeloid cell counts may increase risk of ependymoma.

EPID-05. A CASE SERIES OF METASTATIC GLIOBLASTOMA AT MEMORIAL SLOAN KETTERING CANCER CENTER

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Extracranial metastasis of glioma is a rare event during the progression of this type of brain tumor, often occurring in patients with advanced disease. Though intracranial glioblastoma continues to carry a poor prognosis, extracranial metastasis is a particularly unfavorable factor in survival. We present a case series of 7 patients at Memorial Sloan Kettering Cancer Center from 2003 to 2018 with metastatic extracranial glioblastoma

or gliosarcoma. 5 patients had glioblastoma, 1 patient had gliosarcoma, and 1 patient had ganglioglioma that progressed to gliosarcoma. The age of these patients at diagnosis ranged from 14 to 73, with 6 men and 1 woman in this group. The median overall survival from initial diagnosis and from diagnosis of extracranial metastasis was 13.2 (range 9.2–37.6) and 5 (range 1–6) months, respectively. The most common site of extracranial metastatic disease was bone, with other sites being cervical and thoracic lymph nodes, liver, lung, and soft tissues. 5 patients had symptoms referable to their site of extracranial metastasis, with the most common symptoms being pain and shortness of breath. All patients received initial surgical resection, followed by radiation and temozolomide, with subsequent chemotherapeutic regimens administered that were most appropriate for their individual cases. 1 patient had an Ommaya placed during the course of his illness, 1 patient had a craniectomy for severe cerebral edema, and 2 other patients had dural-based disease. These cases demonstrate several potential risk factors for extracranial metastasis of glioblastoma and gliosarcoma, namely disruption of normal anatomic barriers during surgical resection, intraventricular catheter placement, and sarcomatous dedifferentiation. Next steps with this work include further analysis of the molecular features of extracranial glioblastoma metastases with respect to the site of primary disease, which we anticipate will lead to a better understanding of the molecular mechanisms of metastasis and may improve treatments for these patients.

EPID-06. RACIAL DISPARITY IN THE SURGICAL MANAGEMENT OF SKULL BASE CHONDROSARCOMAS: A SURVEILLANCE, EPIDEMIOLOGY, AND END RESULTS (SEER) ANALYSIS

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INTRODUCTION: Surgical resection is a key aspect in the treatment paradigm for skull-base chondrosarcomas. Here, we examined the practice patterns in the surgical management of skull base chondrosarcomas using the Surveillance, Epidemiology, and End Results (SEER) database. METHODS: We identified patients with skull-base chondrosarcomas in the SEER registry with available information on whether surgery was recommended. Clinical and demographic variables associated with increased odds of recommendation against surgery were analyzed using univariable and multivariable logistic regression. RESULTS: Of 493 patients with skull-base chondrosarcoma in SEER, 446 were recommended to undergo surgery by the treating physician and 38 were not. On univariate analysis, no significant differences were noted between these groups in terms of age, sex, marital status, size of tumor, tumor location, chemotherapy treatment, or radiation therapy treatment. The odds of recommendation against surgery were significantly higher in African Americans relative to Caucasians (OR=4.056, p=0.001). In a multivariate logistic regression model, the association between African American race and recommendation against surgery remained robust (OR 4.28, p=0.01) while the association with median income lost statistical significance (OR=0.966 per $1000 increase in income, p=0.083). CONCLUSION: This SEER analysis revealed notable racial disparity in terms of surgical recommendation for patients suffering from skull-base chondrosarcoma. The odds of recommendation against surgery were significantly higher in African American patients in a multivariate model that accounted for other available, pertinent variables.

EPID-07. RELATIONSHIP BETWEEN COGNITION, SPEED OF PRESENTATION AND SEMANTIC VERBAL FLUENCY TEST IN PATIENTS WITH NEW INTRA-CEREBRAL TUMORS

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Patients with brain tumors often have subtle cognitive change, which may go unnoticed by patient and family doctor. This may result in delays in diagnosis. We studied the relationship between first and subsequent symptoms and semantic verbal fluency test (SVFT), a quick cognitive screening test. (Normal median SVFT for 60–79 age range who have 12 years of education = 17 animals; 10th percentile is 12 animals). Patients with new intrinsic brain tumors had structured patient interviews to determine first and subsequent symptoms. Patients were specifically asked about cognition. A cognitive screening test (SVFT – how many animals can you think of in a minute) was performed in patients as part of the Addenbrookes Cognitive Exam on admission. Symptoms were categorized into focal, non-focal, episodic attack or headache. 127 patients with cerebral brain tumors were assessed– glioma (n=94 HGG=79;LGG=15); metastasis (n= 28); primary CNS lymphoma (n=5). Mean age, 56.2, (SD 13.1): 55% male. 16 patients presented with a solitary symptom (episodic (9); focal (4); headache (3); non-focal (0)). As symptoms accumulated time to diagnosis increased "1 symptom" (med 23 days); "2 symptoms" (med 42 days) ">=3 symptoms" (med 44 days). Mean SVFT was lowest in HGG (mean 10.9) vs LGG (mean 15.7). Patient awareness of cognitive problems did not correlate with SVFT score (aware (n=50) mean 11.9 v unaware (n=77) mean 11.7)) Presentation with an isolated cognitive complaint is uncommon in cerebral brain tumors. Diagnosis becomes apparent when combined with headache or focal symptoms. Diagnosis becomes easier when multiple symptoms accumulate, but there are increasing delays. Many patients with headache also have asymptomatic cognitive problems on SVFT. Where there is a suspicion of cerebral problem e.g. headache suspicious of cancer, it is insufficient just to ask patients whether they have cognitive problems. SVFT may be a helpful red flag.

EPID-08. EFFECT OF HEALTH DISPARITIES ON OVERALL SURVIVAL OF PATIENTS WITH GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GB) is the most common primary malignant brain tumor in adults. It remains uncertain the potential effects of health disparities in this patient population. METHODS: We conducted a retrospective chart review of newly diagnosed GB patients from 2000–2015 at a free standing dedicated cancer center (MD Anderson Cancer Center - MDACC) and a safety net county hospital (Ben Taub General Hospital - BT) located in Houston, Texas. We obtained demographics, insurance status, clinical variables, extent of resection, treatment, and other known prognostic variables (Karnofsky Score – KPS) to evaluate their role on overall GB survival (OS). RESULTS: We identified 1,073 GB patients consisting of 177 from BT and 896 from MDACC. We found significant differences by ethnicity, primary language, insurance status, marital status, KPS at diagnosis, extent of resection, and percentage of patients receiving standard of care (SOC) consisting of concurrent chemoradiation followed by adjuvant temozolomide between the two centers. OS was 1.64 years for MDACC patients and 1.24 years for BT patients (p<0.0176). Of the 177 BT patients, 40 (23%) had KPS KPS <70 at diagnosis, compared to 6 (0.01%) of the 896 MDACC patients. Only 81 (45.8%) of BT patients received SOC compared to 577 (64%) of MDACC patients (p<0.0001). However, there was no significant difference in OS for patients who received SOC, 1.84 years for MDACC patients and 1.99 years for BT patients (p<0.4787). Of the 96 BT patients who did not receive SOC, 29 (30%) had a KPS less than 70 at time of diagnosis and 77 (80%) did not have insurance. CONCLUSIONS: GB patients treated at a safety net county hospital had similar OS compared to a free standing comprehensive cancer center when receiving SOC. County hospital patients had poorer KPS at diagnosis and were often lacking health insurance potentially affecting their ability to receive SOC.

EPID-09. THE ROLE OF GENDER PHENOTYPE IN PAEDIATRIC CNS TUMOUR INCIDENCE AND SURVIVAL

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INTRODUCTION: The impact of patient gender in germinomas and medulloblastomas is a well-documented factor influencing incidence and outcome of these tumours, which may be due to differential susceptibility to tumourogenesis and treatment response between the genders. The role of gender phenotype in other paediatric CNS tumours is not as distinct. METHODS: The Surveillance Epidemiology and End Result (SEER) registry (covering 28% of US patients) between 2000–11 was used to collect age-adjusted incidence and overall survival (OS) rates for common paediatric (<=19 years) CNS tumours - pilocytic astrocytoma (PA), anaplastic astrocytoma (AA), glioblastoma multiforme (GBM), medulloblastoma (MB), sPNET, ependymoma (EP), germinoma (G) and pleomorphic xanthoastrocytoma (PXA). Patients with histologically confirmed, ICD-03 coded, first tumours, were included. Mortality was defined as any cause of death between 1–60 months of diagnosis. Results were statistically analysed by Incidence Rate Ratio (IRR), Kaplan-Meier and Log Rank (Cox Propor-

tional Regression Hazard) Testing (p<0.05). RESULTS: A total of 5712 cases were registered (3169 male, 2543 female). Males exhibited the highest incidence of all tumours except PA. Comparing the opposite gender at the same ages, 1- and 5-year OS was better in males aged 3–4 years for MB, sPNETs, GBM and AA, females aged 0–19 years for MB, females aged 0–2 years and 15–19 years for AA; 5-year OS was better in females aged 0–2 years with EP; 1-year OS was better in males aged 3–4 year with AA. CONCLUSION: Population-based registries such as SEER can deliver credible data and minimise bias. Gender differences in both incidence and OS for different paediatric CNS tumours provide useful prognostic information for clinicians. Further research into possible hormonal and epigenetic differences may provide targets unique to males or females to tailor therapy.

EPID-10. TUMORS IN THE CAUDA EQUINA: A SEER ANALYSIS OF TUMOR TYPES AND PREDICTORS OF OUTCOME

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BACKGROUND: Cauda equina tumors are histologically diverse. Cauda equina has a dedicated site code (C72.1) in the International Classification of Diseases for Oncology (ICD-O3) and is often excluded during analyses of other primary spinal cord tumors. Therefore, less is known about tumors in this location. OBJECTIVE: Using SEER data, we analyzed the demographic features, tumor types, treatment and survival of primary cauda equina tumors. METHODS: SEER data from 1997 to 2015 were reviewed for primary cauda equina tumors (C72.1) excluding the tumors of spinal meninges (C70.1). We describe demographic characteristics of the cohort, tumor types and compared these with clinical outcome using univariate analysis. Treatment and survival analysis was performed using Kaplan-Meier curves. RESULTS: Ninety-two patients with primary cauda equina tumors met the inclusion criteria. These tumors comprised ependymoma (73%), glioblastoma (5%), lymphoma (5%), Ewing sarcoma (3%) and solitary occurrences of neurilemmoma, fibrosarcoma, germinoma, teratoma etc. The median age at diagnosis was 49 years (<1 year and 98 years), 55% were males, predominantly white (87%). Eighty percent of the patients received surgery. Median follow up time was 102.5 months. Of the 92 patients, 68 (73%) are still alive. The cause of death is tumor or CNS related in 58% of the patients. Of the 92 patients, 62 patients (67.4%) survived more than 5 years and 19 patients (20.7%) died before 5 years. 11 patients (12.0%) were censored before 5 years. Using univariate analysis, age at diagnosis was independent predictor of increased tumor specific mortality (HR1.03, CI 1.01–1.06), while non-ependymal tumor type (HR 0.14, CI 0.06- 0.33) and surgical intervention (HR 0.18, CI 0.08- 0.40) were independent predictors of improved survival (p<0.001 for each). CONCLUSION: Cauda equina tumors are predominantly ependymal in origin. Although most patients do well, older age and lack of surgical intervention are associated with worse survival.

EPID-11. PROGRESSION OF IDH MUTANT GLIOMA AFTER FIRST RECURRENCE: DEVELOPMENT OF A FEASIBLE CLINICAL TRIAL ENDPOINT IN THE RECURRENT SETTING

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BACKGROUND: Isocitrate dehydrogenase (IDH) mutant tumors represent a distinct subtype among diffuse gliomas, with improved prognosis compared to grade-matched IDH wild-type tumors. As a basis for clinical trial design of IDH-targeting drugs, we sought to describe outcomes exclusively within the glioma subgroup defined by IDH1 mutation. METHODS: We retrospectively analyzed 275 IDH mutant glioma patients (48.7% grade II and 51.3% grade III tumors; 65.5% astrocytic and 34.5% oligodendroglial tumors) treated at our institution. We calculated progression and survival statistics, including median time to second progression event following first episode of recurrence, using the method of Kaplan-Meier. Estimated survival proportions were correlated with molecular, histologic and clinical factors. RESULTS: During a median follow-up of 6.4 years, 44 deaths (7.6%) and 149 first progression events (54.1%) were observed, with estimated median PFS of 5.7 years (95% CI 4.7–6.4) and median OS of 18.7 years (95% CI 12.2 years - not reached). We validated the effect of grade, molecular diagnosis and treatment paradigms on PFS in our cohort and found results consistent with existing literature. Following the first episode of progressive disease, 79 second progression events occurred during a median follow-up period of 4.1 years. The estimated PFS following first progressive event (PFS-2nd) is 3.0 years (95% CI 2.1–4.1). CONCLUSION: This well-characterized cohort of IDH mutant glioma patients demonstrate progression and survival outcomes reflecting published literature and serves as a reasonable historical control population. Notably, the PFS interval accelerates during disease course, with interval between first to second recurrence (3.0 years) shorter than time from diagnosis to first recurrence (5.7 years). The novel survival statistic PFS-2nd offers an accurate and relevant surrogate outcome for the design of clinical trials investigating experimental drug efficacy at recurrence. These findings highlight that inappropriate comparison to historical baseline PFS may result in prematurely abandoning promising agents.

EPID-12. USING GERMLINE VARIANTS TO PREDICT GLIOMA RISK AND IDENTIFY GLIOMA SUBTYPE PRE-OPERATIVELY

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To date, 25 single nucleotide polymorphisms (SNPs) have been shown to be associated with overall glioma risk or with risk of specific subtypes of glioma. We hypothesized that the inclusion of these 25 SNPs with patient age at diagnosis and sex could predict risk of glioma as well as predict IDH mutation status. Thus, case-control design and multinomial logistic regression were used to develop models to estimate the risk of glioma development while accounting for molecular subtypes. Case-case design and logistic regression were used to develop models to predict IDH mutation status. Each model included all 25 glioma risk SNPs, patient age at diagnosis and sex. A total of 1273 glioma cases and 443 controls from Mayo Clinic were used in the discovery set, and 852 glioma cases and 231 controls from UCSF were used in the validation set. All samples were genotyped using a custom Illumina OncoArray. We observed that patients in the highest 5% of the risk score had more than a 14-fold increased relative risk of developing an IDH-mutant glioma, compared to patients with median risk score. Large differences in lifetime absolute risk were observed at the extremes of the risk score percentile categories. For both IDH-mutated 1p/19q non-codeleted glioma and IDH-mutated 1p/19q-codeleted glioma, the lifetime risk increased from almost null to 2.3% and almost null to 1.7%, respectively. The SNP-based model that predicted IDH mutation status had a validation c-index of 0.85. These results suggest that germline genotyping has the potential to provide a new tool for clinicians for the initial management of newly-discovered brain lesions. Specifically, given the low lifetime risk of glioma, SNP-based risk scores should not be useful for general population screening. However, with further research these risk scores may be useful in certain clinically-defined high-risk groups.

EPID-13. ANTI-ANGIOGENIC THERAPY FOR HIGH-GRADE GLIOMA: A META-ANALYSIS

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BACKGROUND: We have previously published a Cochrane meta-analysis of the efficacy of anti-angiogenic therapy (AAT) in high grade glioma (HGG) in 2014. Since this time, a number of key trials have been published. An updated meta-analysis was performed to account for these new results. METHODS: The primary analysis was to evaluate the pooled overall survival (OS) of AAT in HGG and the secondary analysis was to evaluate the pooled progression-free survival (PFS) of AAT in HGG. Subgroup analyses were performed according to treatment setting and AAT combined with chemotherapy versus chemotherapy alone. Searches were conducted to identify randomised controlled trials (RCTs) including CENTRAL, MEDLINE and Embase to August 2017. Proceedings of oncology conferences and trial registries were also searched. RESULTS: 11 eligible RCTs were identified (N=3743). There was no improvement in OS with the addition of AAT (pooled hazard ratio (HR) of 0.95, 95% confidence interval (CI) 0.88, 1.02; p=0.16) overall, or in the adjuvant or recurrent settings (HR 0.93, 95% CI 0.86, 1.02; p=0.12 and HR 0.99, 95% CI 0.85–1.16; p = 0.90). Pooled analysis of OS for AAT with chemotherapy compared to chemotherapy also did not show an improvement (HR 0.92, 95% CI 0.85–1.00; p=0.05). Pooled analysis of PFS from ten studies showed improved PFS with AAT (HR 0.73; 95% CI 0.68, 0.79; p < 0.00001). These improvements in PFS occurred in the adjuvant (HR 0.75, 95% CI 0.69–0.82; p < 0.00001) and recurrent settings

(HR 0.64, 95% CI 0.54–0.76; p< 0.00001) and when AAT was combined with chemotherapy compared to chemotherapy alone (HR 0.72, 95% CI 0.66–0.77; p <0.00001). CONCLUSIONS: The use of anti-angiogenic therapy does not improve survival in newly diagnosed people with GBM, despite improved progression free survival. There is no evidence of a survival advantage for anti-angiogenic therapy over chemotherapy in recurrent GBM.

EPID-14. ECHOES FROM THE PAST- AN HISTORICAL COHORT STUDY OF LOW DOSE RADIATION FOR TINEA CAPITIS AND BRAIN TUMOR INCIDENCE IN ISRAEL

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Ionizing radiation is the only established environmental risk factor for brain tumors. Treatment with low dose radiation was the standard of care for treatment of tinea capitis till 1960 and was given in Israel mainly to children immigrating to Israel from North Africa and the Middle East between 1948–1960. The aim of this study was to evaluate the influence of treatment with low dose radiation on the epidemiology of brain tumors in Israel. This study analyzed two cohorts of Jewish men and women who underwent health examinations at the age of 17 years between 1967 and 1991 and were followed by linkage to the national cancer registry up to 2012. The first cohort included 376,336 participants born in 1948–1959 and the second cohort included 474,923 participants born in 1960–1971. The incidence of all brain tumors was 541 in the first cohort [crude rate (CR) 5.14] and 629 in the second cohort (CR 4.72). There was a significant decrease in the rate of meningioma in the second cohort [CR in the second cohort 1.36 expected CR 1.67 (p=0.041)]. In the first cohort there was important impact to origin-with higher incidence among those from north Africa and Middle East. This effect was ablated in the second cohort that included participants who had not been exposed to radiation treatment. The effect of origin was mainly evident in the incidence of all brain tumors grouped together (p=0.0003), meningiomas (p=0.00015) and cranial nerve tumors (p=0.006) and was strongest among those originating from North Africa. Our study reveals that treatment with low dose radiation for tinea capitis- appears to have affected the incidence of meningiomas in the cohort exposed to irradiation and dramatically changed the association of ethnicity with brain tumors in Israel, an effect that disappeared once treatment with low dose radiation was halted.

EPID-15. INCIDENCE PATTERNS OF PRIMARY BRAIN AND OTHER CENTRAL NERVOUS SYSTEM TUMORS IN APPALACHIA

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BACKGROUND: The Appalachian region is a large geographic and economic region in the United States (US), representing 7.69% of the US population. This region is more rural, whiter, older, and residents are more likely to be under the poverty line as compared to the rest of the US. Limited research has been done on primary brain and other CNS tumor epidemiology in this region. In this analysis we characterized their incidence patterns. METHODS: Data from 2006–2015 were obtained the Central Brain Tumor Registry of the US. Appalachian counties were categorized using the Appalachia Regional Council scheme. Overall and histology-specific age-adjusted incidence rates per 100,000 population were generated by region, sex, race, and age groups. RESULTS: Overall incidence within Appalachia was 22.62 per 100,000, which is not significantly different from the rest of the US (22.77, p=0.1189). Malignant incidence was 5% higher in Appalachia (7.55/100,000 as compared to 7.23/100,000, p<0.0001), while non-malignant incidence was 3% lower (15.07/100,000 as compared to 15.51/100,000, p<0.0001). The largest differences in histology-specific incidence were in oligodendroglioma, where non-Appalachian incidence was 24% higher (p<0.0001), and hemangioma, where Appalachian incidence was 20% higher (p<0.0001). Among Blacks, incidence of both malignant and non-malignant tumors was 8% lower in Appalachia as compared to the rest of the US (p<0.0001). Incidence was also lower in White Hispanics, where malignant incidence was 19% lower, and non-malignant incidence was 30% lower (p<0.0001). CONCLUSION: Evaluating place-based health disparities is critical to understanding population variation in incidence. Appalachian counties have increased malignant and decreased non-malignant brain tumor incidence as compared to the rest of the US. Incidence of non-malignant tumors was lower among all racial/ethnic groups, with the largest differences in Blacks and White Hispanics. These differences may be attributable to access to health care.

EPID-16. CONSIDERATIONS FOR A SURGICAL RCT IN LOW-GRADE GLIOMAS: A SURVEY

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BACKGROUND: The management of diffuse low grade gliomas (LGGs) has seen a paradigmatic shift favoring maximal safe surgical resection (MSR). While this approach is not based on randomized-controlled trials (RCTs), the extent of evidence from observational data has prompted arguments against equipoise in LGG management, thus suggesting an RCT comparing MSR with other surgical options unethical. OBJECTIVE: To explore opinions within the SNO neuro-oncology community regarding feasibility, ethics, and endpoints for a putative RCT comparing MSR with other management options. METHODS: A survey of 19 questions was developed on the Qualtrics® platform and distributed to the SNO members on a one-time basis. RESULTS: Among 128 participants, 111 (87%) were consultants. The majority were neuro-oncologists (70, 55%), followed by neurosurgeons (41, 32%), and radiation oncologists (7, 6%). Thirty-five of 111 (32%) thought there was equipoise in LGG management. An RCT was thought to be ethical in 56/116 (48%) and potentially feasible in 66/108 (61%). Potential willingness to participate in an RCT was expressed by 73/108 (68%). There were no correlation between sub-specialty and any of the responses. The ideal patient for randomization was thought to be age < 40 years with minimal neurological symptoms presenting with a small (0-3cm) non-enhancing lesion in an eloquent/deep location. The ideal endpoint selected by most 39/97, 40% was the combination of overall survival and quality of life. CONCLUSION: The majority of respondents ruled out equipoise. However, LGGs are heterogeneous and an RCT may be needed to define the ideal management approach in a specific subset of this population. Quality of life and other patient-centered parameters must be incorporated as primary endpoints, alongside survival. Due to anticipated challenges such as a small sample size, heterogeneous management approaches, and a protracted clinical course, careful consideration of feasibility and a large-scale, multi-center approach would be necessary.

EPID-17. PRIMARY LYMPHOMA OF THE CENTRAL NERVOUS SYSTEM. CLINICAL AND THERAPEUTIC EXPERIENCE

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BACKGROUND: Primary lymphoma of the central nervous system (PCNSL) is a non-Hodgkin lymphoma variety representing 4–5% of intracranial tumors and 5% of all lymphomas. It originates in the encephalon, eyes, leptomeninges and spinal cord without evidence of lymphomatoid activity at the systemic level; the subtype of lymphoma is mostly type B cells. MATERIAL AND METHODS: A descriptive study was carried out on patients diagnosed with PCNSL who were treated in 3rd level centers in Mexico between the years 1980–2016. We included patients with scrutiny for the search for systemic lymphoma. The results were analyzed by simple frequencies and in the case of disease free time (DFT) and Global survival (GS) was analyzed by Kaplan Meier curves and the differences between curves by Log Rank. RESULTS: In a total of 215 cases only 74 cases had PCNSL. 45% were women and 55% were men. 36.7% were over 60 years old. All the patients were HIV negative. The most frequent clinical manifestations were motor deficit (60%) and cognitive alterations (52%). The majority of patients received some form of chemotherapy (89%). The treatment was mostly based on Metrotexate at High doses. The DFT was: Palliative 2 months, Chemotherapy alone 16 months, Radiation therapy alone 27 months and Radiochemotheraphy 35 months. Of the patients receiving chemotherapy alone, 7 patients received CHOP, 7 patients MTX, TMZ and Cytarabine (META protocol), 21 patients only MTX, and 9 as a combination of MTX and cytarabine, with the META protocol having the longest survival. The only significant factor for radiological response and clinical prognosis was the combined use of radiochemotherapy (p = 0.044). CONCLUSION: Lymphoma represents a tumor pathology with a high clinical radiological response to treatment, although the response is not durable. Its early identification and multidisciplinary management is fundamental for the best outcome in these patients.

EPID-18. MALIGNANT PRIMARY BRAIN AND OTHER CENTRAL NERVOUS SYSTEM TUMOURS DIAGNOSED AMONG THE CANADIAN POPULATION FROM 2009 TO 2013

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The Canadian Brain Tumour Registry (CBTR) project was established with the aim of enhancing infrastructure for surveillance and clinical research to improve health outcomes for brain tumour patients in Canada. We present a national surveillance report on malignant primary brain and central nervous system (CNS) tumours diagnosed in the Canadian population from 2009–2013. Patients were identified through the Canadian Cancer Registry

(CCR); an administrative dataset that includes cancer incidence data from all provinces/territories in Canada. Cancer diagnoses are coded using the ICD-O3 system. Tumour types were classified by site and histology using The Central Brain Tumour Registry of the United States definitions. Incidence rates (IR) and 95% confidence intervals (CI) were calculated per 100,000 persons and standardized to the 2011 census population age distribution. Overall, 12,115 malignant brain and CNS tumours were diagnosed in the Canadian population from 2009–2013 (IR:8.43; 95%CI:8.28,8.58). Of these, 6,845 were diagnosed among males (IR:9.72; 95%CI:9.49,9.95) and 5,270 among females (IR:7.20; 95%CI:7.00,7.39). The most common histology overall was glioblastoma (n=5,830; IR:4.06; 95%CI:3.95,4.16). Among those aged 0–19 years, 1,130 malignant brain and CNS tumours were diagnosed from 2009–2013 (IR:3.36; 95%CI:3.16,3.56). Of these, 625 were diagnosed among males (IR:3.32; 95%CI:3.34,3.92) and 505 among females (IR:3.08; 95%CI:2.81,3.36). The most common histology among the paediatric population was pilocytic astrocytoma (n=245; IR:0.73; 95%CI:0.64,0.83). The presentation will include: IRs by histologies, the geographic distribution of cases, an analysis of incidence trends over time for major histological groups and a discussion of progress in obtaining surveillance information on nonmalignant tumours.

EPID-19. BRAINSTEM OBESITY IN PEDIATRIC GANGLIOGLIOMA

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INTRODUCTION: Hypothalamic obesity is a well-known entity. Here we present, a case of a pediatric patient with brainstem ganglioglioma who developed rapid-onset obesity, along with relevant literature review. RESULTS: A 3-year-old girl presented with progressive head tilt, ophthalmoplegia, ataxia and slurry speech. MRI brain revealed locally expansile, enhancing tumor involving brainstem. MR tractography revealed tumor infiltrating corticospinal tracts. Tumor biopsy confirmed ganglioglioma WHO grade I, BRAF v600E mutated. The patient was started on a targeted chemotherapy of BRAF inhibitor vemurafenib. Body mass index (BMI) was way above 99 percentile for age with familial obesity and peri-operative use of dexamethasone contributing to it. Review of the growth chart dating back to a year before diagnosis revealed that the patient's weight had exponentially increased from 32 to 52 pounds (95 to above 99 percentile for age), coinciding with the patient's progressive neurological symptoms. Endocrine workup to investigate obesity did not reveal pituitary or hypothalamic dysfunction. The patient has shown a sustained clinical and radiographic response to chemotherapy 6 months post-diagnosis. Despite adherence to dietary recommendations, the patient's weight continues to increase (60 pounds). CONCLUSION: In our patient, obesity appears to be primarily related to the brainstem tumor causing permanent disruption of circuits running between the brainstem and hypothalamus. DISCUSSION: Studies have shown that brainstem neuronal circuits (involving the nucleus of solitary tract) are sensitive to short-term energy regulation and that the central melanocortin pathway connecting neurons in brainstem and hypothalamus plays a role in regulating body weight. An adult patient with brainstem cavernoma was reported to have postoperative hyperphagia and progressive obesity possibly from injury to the hypothalamic circuits connecting brainstem to the hypothalamus. Further clinical research is warranted to validate brainstem obesity as a clinically significant entity.

EPID-20. THE SURVIVAL BENEFIT OF VALPROIC ACID IN GLIOBLASTOMA TRENDS AWAY FROM SIGNIFICANCE WITH NEWER STUDIES: A SYSTEMATIC REVIEW AND META-ANALYSIS

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BACKGROUND: Glioblastoma (GBM) is a dismal disease that presents often with seizure. The use of anti-epileptic medication valproic acid (VPA) has been suggested to provide survival benefit. This study aims to quantify the survival benefit of VPA in GBM on overall survival (OS) and determine if a trend in reported effect over time exists. METHODS: Searches of 7 electronic databases from inception to April 2018 were conducted following PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines. There were 1498 articles identified for screening. Prognostic hazard ratios (HRs) derived from multivariate regression analysis were extracted and analyzed using meta-analysis of proportions and linear regression. RESULTS: Seven observational studies reporting prognostic HRs satisfied selection criteria. They described 3631 primary GBM diagnoses, with 603 (17%) receiving VPA in their treatment. VPA was shown to confer a statistically significant OS advantage the pooled cohort (HR, 0.709; 95% CI, 0.551–0.914; I^2=60.1%; p=0.008) without specific bias concerns. However, this survival advantage trended towards null significance in newer studies (effect coefficient, 1.151; p=0.023). CONCLUSIONS: The current literature suggests that VPA confers a significant, prognostic OS advantage in GBM independent of other prognostic factors. This may be through a variety of possible biological mechanisms and clinical consequences. However, newer studies are significantly trending away from such a positive association, towards null significance. Larger, prospective randomized controlled studies are needed to validate if VPA itself provides true OS benefit in GBM patients.

EPID-21. PREDIAGNOSTIC PRESENTATIONS OF GLIOMA IN PRIMARY CARE: A CASE-CONTROL STUDY

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BACKGROUND: Little is known about symptoms and signs of glioma patients in the year(s) before diagnosis. This study aimed to assess the prevalence of clinical symptoms glioma patients may present with to the general practitioner (GP) in the five years prior to diagnosis, and whether these can be distinguished from patients with (1) other central nervous system (CNS) disorders or (2) any other condition. METHODS: Glioma patients were matched to controls based on age, sex and diagnosis date, using anonymized GP registries. Prevalences of nine prediagnostic symptoms (seizures, headaches, motor impairments, cognitive/mental impairments, visual disorders, mood disorders/fear, general symptoms, sensory complaints and metabolic/endocrine symptoms) were evaluated in the five years prior to diagnosis, and divided into three time periods: 60-24 months, 24–6 months and ‹6 months. RESULT: Thirty-six glioma patients were matched with 72 control patients. In total, 2491 (range 0–102) visits to the GP were analyzed. There were no significant differences between groups in the number of visits over 5 years (711 versus 989 versus 791 in glioma, CNS and other patients respectively; p=0.381), or in each of the three time periods (data not shown). The prevalence of motor symptoms in the period 60-24 months was significantly higher in the CNS patients as opposed to the other groups (4 versus 0 in both groups; both p=0.039). Moreover, ‹6 months prior to diagnosis CNS patients differed significantly in mood disorders/fear compared to other controls (8 versus 0, p=0.012), but not glioma patients versus CNS patients (5 versus 8, p=0.816) or glioma versus other patients (p=0.221). CONCLUSION: In this sample, glioma patients could not be distinguished from CNS or 'other' control patients with respect to the number of GP visits before diagnosis or prediagnostic symptoms. Nevertheless, when mood disorders occur, all CNS disorders should be considered by the GP, including glioma.

EPID-22. RACIAL AND ETHNIC DIFFERENCES IN ADULT GLIOMA INCIDENCE AND SURVIVAL IN THE UNITED STATES

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BACKGROUND: Glioma is the most commonly occurring malignant brain tumor in the United States (US), and incidence and survival varies by age, sex, and race/ethnicity. The goal of this analysis was to estimate the racial and ethnic differences in glioma incidence and survival by histologic subtype. METHODS: Data were obtained from the Central Brain Tumor Registry of the US (incidence data from ~99.9% of the US), and the Surveillance, Epidemiology and End Results program (survival data for ~28% of the US). Histology-specific age-adjusted incidence rates were generated by race, Hispanic ethnicity, sex, and age groups. Histology-specific relative survival rates were generated by race, Hispanic ethnicity, treatment type, insurance status, and age. RESULTS: Overall incidence of glioblastoma, non-glioblastoma astrocytoma, and oligodendroglial tumors were higher among White non-Hispanic (WNH) individuals than among any other group. Most tumors were more common in males than in females across all race/ethnic groups. The majority of tumors occurred in those aged 40+ years, with differences in incidence by race/ethnicity appearing in all three age groups. Survival after diagnosis was similar among non-WNH individuals, but was lower among WNH individuals for glioblastoma, irrespective of treatment. Survival after diagnosis with glioblastoma among those that receive chemo-radiation remains higher in non-WNH groups after adjustment for age and extent of resection. CONCLUSION: US. Incidence and survival patterns of most glioma histologies varied significantly by race/ethnicity, with WNHs having higher incidence and lower survival compared to individuals of other racial and ethnic groups. Further examination is necessary in order to determine the factors contributing to these differences.

EXPERIMENTAL THERAPEUTICS

EXTH-01. MODELING THE SAFETY OF TOPICAL AGENTS FOR SKIN TOXICITY ASSOCIATED WITH TUMOR TREATING FIELDS THERAPY IN GLIOBLASTOMA

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Tumor Treating Fields are low intensity, intermediate frequency, alternating electric fields that are approved for the treatment of patients with glioblastoma (GBM). TTFields are applied continuously using a set of four transducer arrays placed on the skin. The main TTFields-related adverse event is mild-moderate dermatitis occurring under the arrays, which is primarily caused by long-term exposure to irritants and may be exacerbated by local hyperhidrosis and occlusion. Strategies that mitigate skin irritation may improve patients' quality of life and compliance with TTFields. Four product groups (antiperspirants, moisturizing creams, antibiotics, and skin barriers) have been anecdotally reported to alleviate factors related to TTFields-related skin irritation. Nonetheless, not all of the aforementioned products are compatible with TTFields as some may increase electrical impedance, which may lead to increased temperatures beneath the arrays. This study investigated the effect of applying 40 commercially available skin care products on electrical impedance during TTFields application, using a rat model. In these experiments, 200 kHz TTFields were applied using ceramic disks and hydrogels identical to those used with human arrays. Changes in impedance caused by applying each product on the rat skin were measured using the Optune device and an LCR meter system. The review identified a set of skin care products from all 4 groups that did not lead to a significant change in the impedance. Preliminary tests in the clinical setting with these products were promising in a few patient case studies. These results suggest that local application of TTFields-compatible skin care products could maintain normal electrical resistance of the scalp-array interface, thereby allowing optimal delivery of TTFields to the brain. These products should be prospectively investigated for their potential role in minimizing TTFields–related skin irritation.

EXTH-02. DUAL DRUG MODIFIED NANOVESICLES FOR ENHANCING THE THERAPEUTIC EFFICACY OF DOXORUBICIN TOWARDS GLIOBLASTOMA AND MPNST

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Doxorubicin is a topoisomerase II inhibitor demonstrated to be effective in several cancers including malignant peripheral nerve sheath tumors (MPNST) and glioblastoma in cell culture, but at higher dose causes adverse effects like cardiotoxicity in patients. This minimizes the therapeutic dosage of doxorubicin administered for such cancers. To overcome this issue we have developed novel lipid based nanovesicles carrying doxorubicin with certain chemosensitizing agents like farnesyl thiosalicylic acid (FTS), a Ras inhibitor and dexrazoxane (DEX) in the lipid bilayer component of the nanovesicles using a microfluidic encapsulation method. FTS modified nanovesicles with doxorubicin in the core demonstrated an enhanced cytotoxic potential in glioma cells and neurofibromatosis type-1 associated MPNST cells (NF-1 MPNST). To determine the in vivo therapeutic efficacy, U87-Luc subcutaneous tumor bearing mice were injected twice weekly with 4mg/kg body weight dose of FTS-LIP-DOX or LIP-DOX (n=8) for 6 weeks. After 6 weeks of treatment, the median tumor volume in the control group reached 3212 mm^3 and that of LIP-DOX and FTS-LIP-DOX reached a mean volume of 111 mm^3 and 64.4 mm^3 respectively. In a second formulation, doxorubicin was combined with dexrazoxane (DEX), a metal chelator and topoisomerase inhibitor in a liposome formulation. In our pilot study *in vivo* investigation we treated mice with DOX-DEX-liposomes(n=3) or DOX-liposomes (n=2) at a dose of 7 mg/kg body weight with respect to doxorubicin, twice weekly for 5 weeks in a MPNST mouse model. In the first 3 weeks, a slower tumor progression in the DOX-DEX liposomes group was observed compared to DOX-liposomes formulation, beyond which the tumor progression in both groups approached each other. We didn't observe any adverse effect based on serum chemistry analysis. This novel therapy provides an opportunity for doxorubicin dose escalation in MPNST mouse model with improved therapeutic efficacy.

EXTH-03. LOCAL ONCOLYTIC ADENOVIRUS TREATMENT AFFECTS BOTH THE INNATE AND ADAPTIVE ARMS OF THE IMMUNE SYSTEM AND PROVIDES AN AVENUE FOR ENHANCING IMMUNOTHERAPIES FOR GBM

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The oncolytic adenovirus (OV) Delta24-RGD, also known as DNX-2401, is currently under clinical investigation for GBM. We studied multiple components of the immune system in response to local treatment with this agent using both *in vitro* and *in vivo* models and patient-derived materials. METHODS: C57BL/6 mice bearing orthotopic GL261 tumors were treated with Delta24-RGD virus. Tumors were analyzed for cytokine levels, influx of immune cells, and Tcell expression of a panel of co-signaling molecules by flowcytometry. Combination virotherapy with checkpoint inhibitors was tested *in vitro* and *in vivo*. Effects of Delta24-RGD infection on tumor macrophages was studied in co-cultures of human monocyte-derived macrophages analyzed for immunophenotype and cytokine secretion. CSF from Delta24-RGD-treated patients was analyzed for immune cell infiltrates and cytokine levels. Tumor macrophages in resected material from one patient were immunophenotyped. RESULTS: Analysis of OV-treated GL261 tumors revealed a rapid increase in inflammatory cytokines followed by an influx of macrophages, CD4+ and CD8+ Tcells. Analysis of co-signaling molecules on intratumoral Tcells revealed marked changes in ICOS and PD-1 expression. *Ex vivo* functional assays identified PD-1 blocking antibodies as an effective enhancer of OV-mediated anti-tumor immunity, which was confirmed *in vivo*. Macrophages co-cultured with OV-infected GBM cells displayed a shift from M2 to M1 phenotype and pro-inflammatory cytokine production. This shift was dependent on the presence of large secreted molecules from OV-infected tumor cells. CSF from Delta24-RGD-treated GBM patients contained increased levels of all Tcell subsets and cytokine levels indicative of a pro-inflammatory microenvironment. Macrophages in resected tumor from one patient showed significantly greater M1 characteristics than from control GBM tissue. CONCLUSION: Together, these *in vitro, in vivo* and patient studies contribute to our understanding of the complex interactions that occur between the tumor and the different arms of the immune system in local oncolytic virus treatment.

EXTH-04. TARGETING CD97 BY NOVEL FUSION PROTEIN DAF-FC INHIBITS GBM INVASION AND INDUCES ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY

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INTRODUCTION: The poor prognosis of GBM reflects its capacity to supplement proliferative programs with pro-tumoral interactions with the microenvironment, including invasion and immunosuppression. There is a need to simultaneously target these processes, as they can be temporally, mutually exclusive phenotypes toggling back and forth. We have shown CD97 to be upregulated in GBM and capable of mediating each of these pro-tumoral functions in GBM by binding three ligands: decay accelerating factor (DAF/CD55), chondroitin sulfate, and integrins. To validate CD97 as a therapeutic target in glioblastoma, we partnered with an industry collaborator to produce a DAF-Fc fusion protein in a plant expression system combining DAF with IgG1 Fc to block CD97. METHODS: CD97 was targeted with siRNA or DAF-Fc. Scratch and matrigel invasion assays were used to assess migration and invasion, respectively. Antibody dependent cellular cytotoxicity (ADCC) assays used NK-92 human natural killer cells as effector cells and GBM or peripheral blood mononuclear cells (PBMCs) as target cells. Intracranial implantation of glioblastoma cells into athymic mice was followed by DAF-Fc or IgG treatment, with IgG1-Fc detected by immunohistochemistry. RESULTS: CD97 expression was elevated in GBM cells versus astrocytes and lymphocytes, and CD97 knockdown by siRNA reduced migration and invasion by 65–75% ($p<0.01$). Treatment of human and mouse GBM cells with human and mouse DAF-Fc inhibited migration and invasion by 60–80% ($p<0.001$). There was increased ADCC against GBM cells with increased DAF-Fc concentration and effector to target ratio ($p<0.001$), but negligible PBMC toxicity at the highest DAF-Fc dose. Systemically delivered DAF-Fc crossed the blood-brain barrier in orthotopic GBM models with specific tumoral uptake compared to normal brain. CONCLUSIONS: Our results validate DAF-as a GBM therapeutic with negligible off-target effects against normal astrocytes or PBMCs. Further preclinical evaluation in invasive murine models ahead of a phase I trial is underway.

EXTH-05. MicroRNA-138 SUPPRESSES GLIOBLASTOMA PROLIFERATION AND SENSITIZES CHEMOTHERAPY THROUGH FOCAL ADHESION KINASE INACTIVATION

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Glioblastoma is one of the most deadly cancers and limited to only a few therapeutic options that are available in clinical settings, mainly due to the resistance to many chemotherapies. MicroRNAs are increasingly implicated in the tumor survival from chemotherapies, which may lead to a better therapeutic option by overcoming the resistance. Here, we found that miR-138 is significantly downregulated in human glioblastoma patient tissues compared to normal brain tissues. MiR-138 has been known to play as a tumor suppressor in many types of cancer, which may imply that miR-138 can benefit in the development of anti-glioblastoma therapy. Global proteomic analysis revealed that ectopic expression of miR-138 in patient derived glioblastoma primary cells suppressed FAK pathway, which has been known to be highly activated in many cancers including glioblastoma. FAK inactivation by miR-138 resulted inhibition of glioblastoma cell proliferation in vitro and improved tumor free survival in vivo. We also found that miR-138 induced FAK inhibition sensitizes glioblastoma cells to tyrosine kinase inhibitors, such as Imatinib. The studies are indicative of the clinical benefit of miR-138 based therapy of primary and even recurrent glioblastoma.

EXTH-06. CD38-TARGETED THERAPY IN GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) remains a malignancy with a dismal prognosis in spite of current optimal management, including maximal resection, radiation therapy, and alkylating chemotherapy. Relapse is inevitable with the paucity of further effective therapies. The development of novel targeted therapeutics is of prime importance. One such target is CD38, a transmembrane glycoprotein, partakes in receptor-mediated adhesion and cell signaling. It is expressed on GBM microglia/macrophages microenvironment. *In vivo* studies have shown decreased tumor growth and increased survival in CD38 genetic knockout mice, as well as with the use of K-rhein. Daratumumab (DARA) is an FDA-approved IgG1 kappa human monoclonal antibody, with the ability to penetrate blood brain barrier, that upon binding to CD38-bearing tumor cells lead to apoptosis, immune-mediated cell death and downregulation of the immunosuppressive environment. Hence, we wished to see if DARA could enhance efficacy of GBM directed therapies. METHODS: We used flow cytometric analysis to identify CD38 expression on human GBM cells. MTS viability assay is used to detect IC50 of DARA. In addition, Annexin V/PI dual staining is used to detect apoptosis. Further, antibody-dependent cell mediated cytotoxicity (ADCC) and complement mediated cytotoxicity (CDC) assays are used. Additionally, evaluated the effect of DARA on cytotoxicT-cell proliferation. RESULTS: Our *in vitro* data shows that 9% of human GBM cells express CD38 with 250 MFI. IC50 of DARA+DMSO was 150µg/ml vs 75µg/ml with DARA+ temozolomide (TMZ). MTS assay revealed apoptosis after treatment with DARA+TMZ (44.25 ± 1.05%)> DARA (36.33 ± 0.87%) >TMZ (26.59 ± 0.93%, $p<0.05$) in comparison with control(C, 5.359%). % specific lysis via ADCC assay: TMZ+DARA (56.74 ± 2.84%) > DARA (35.30 ± 4.12%) > TMZ (20.57 ± 4.89%), vs C (4.30 ± 1.74%). % specific lysis via CDC with DARA (70.28 ± 9.28%) > TMZ (44.49 ± 0.72%, $p<0.05$), vs C (7.07 ± 0.72). Cytotoxic CD8+ T-cell proliferation was induced with DARA (59.29%) >TMZ (40.47%) > TMZ+DARA (45.82%), vs C (1.43%). CONCLUSIONS: Hence, CD38 is a potential target in GBM that if targeted with DARA +/- TMZ will generate direct anti-GBM effect as well as modulate the tumor microenvironment.

EXTH-07. DESIGN AND EVALUATION OF WP1122, AN INHIBITOR OF GLYCOLYSIS WITH INCREASED CNS UPTAKE.

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Glioblastoma (GBM), an aggressive primary brain tumor, relies on anaerobic glycolysis to produce energy. A known inhibitor of glycolysis, 2-deoxy-D-glucose (2-DG) has been clinically tested but its poor drug-like characteristics limited its practical application for cancer treatment. To overcome this problem, we have performed latentiation of 2-DG. Chemical modification of biologically active 2-DG led to the formation of prodrugs with improved pharmacokinetic and pharmacodynamic properties and subsequently to selection of the lead compound WP1122 (3,6-di-O-acetyl-2-deoxy-D-glucose). Unlike 2-DG, its prodrug WP1222 enters cells and cross blood-brain barrier by passive diffusion rather than by specific glucose transporter, then undergoes deacetylation by esterases and is trapped inside the cell after phosphorylation at C-6 hydroxyl group. 6-phospho-2-deoxy glucose acts as a competitive inhibitor of hexokinase (HK) blocking phosphorylation of D-glucose and subsequently inhibiting glycolytic pathway. Our *in vitro* experiments confirmed inhibition of glycolysis in U87 cells and high sensitivity of broad spectrum of cancer cells to WP1122 both in hypoxic and normoxic conditions (IC_{50} range from 1–10 mM). *In vivo* studies showed that WP1122 is well tolerated by animals even with prolonged exposure and extend survival of mice in orthotopic U87 GBM model. Initial pharmacokinetic experiments demonstrated rapid uptake of WP1122 after oral administration allowing to achieve two orders of magnitude higher maximum concentration of 2-DG in plasma, compared to animals treated with an equal molar dose of pure 2-DG. We also observed significantly higher levels of 2-DG in brains of mice treated with WP1122 than in mice receiving equal dose of 2-DG. In summary, WP1122 is a biologically effective prodrug of 2-DG with a good toxicity profile and promising pharmacokinetic characteristics that warrants detail preclinical and clinical development as a potential therapeutic agent for glioblastomas and other highly glycolytic tumors.

EXTH-08. MESENCHYMAL GLIOBLASTOMA CONSTITUTES A MAJOR ceRNA SIGNATURE IN THE TGF-PATHWAY

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ceRNA networks play important roles in post-transcriptional regulation. Their dysregulation is common in cancer. However, ceRNA signatures are barely examined for mesenchymal glioblastoma characteristic of invasive and aggressive phenotypes. Here, we investigated the mRNAs in ceRNA networks (micNET) of glioblastoma by constructing a GBM ceRNA network, followed by being integrated with STRING protein interaction network. We report that six micNETs (TGFBR2, RUNX1, PPARG, ACSL1, GIT2 and RAP1B) signify mesenchymal GBM across multiple datasets. Patients with highly expressed micNET markers have poor response to TMZ chemotherapy. Mechanistically, the ceRNA interaction were identified between micNETs and miR181s family members. The inhibitor of TGFBR2, LY2109761, demonstrated tumor-suppressive effect on both primary cultured cell and PDX intracranial model. Thereby, the micNETs provide a promising translational significance in diagnosis of mesenchymal GBM as well as novel therapeutic targets. In conclusion, our study revealed that the ceRNA signature of mesenchymal glioblastoma was enriched in the TGF-β pathway and characterized this subtype in the mRNA-miRNA dimension. We identified that key micNETs signatures could be used to diagnose mesenchymal GBM and discovered that the TGF-β pathway is a potential therapeutic target for mesenchymal glioblastoma.

EXTH-09. DIANHYDROGALACTITOL (VAL-083) HAS THE POTENTIAL TO OVERCOME MAJOR CHALLENGES IN THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG)

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OBJECTIVE: VAL-083 is a structurally unique bi-functional DNA targeting agent that readily crosses the blood-brain barrier and accumulates in brain tumor tissue. Herein we assess the activity of VAL-083 as single agent as well as in combination regimens including Wee1 Kinase inhibitor AZD1775 and radiation therapy in patient derived model systems of DIPG. METHODS: DIPG derived cell lines SF8628 and NEM157 (H3.3K27) as well as SF10693 (H3.1K27M) and pediatric glioblastoma cell lines SF188 (H3.3K27 wildtype) were treated with increasing concentrations of single agent VAL-083 as well as in combination with AZD1775 and radiation therapy. To determine potential synergistic activity, we applied the Chou-Talalay method, which allows the quantitative determination of drug interactions by calculating a combination index (CI). *In vivo* activity of VAL-083 as single agent as well as in combination with AZD1775 was assessed in an orthotopic engraftment model of pediatric DIPG (SF8628). RESULTS: The IC_{50} of VAL-083 ranged from 2.1uM to 19.7uM. The combination of VAL-083 and AZD1775 showed synergistic activity in all tested cell lines with CI ranging from 0.405 to 2.066 with < 1 indicating synergy whereas VAL-083 combined with radiation therapy led to only additive effects. Initial in vivo study showed that combined treatment with VAL-083 and AZD1775 conferred greater survival benefit to mice with engrafted DIPG tumors compared to control as well as single agent treatment. Day 49 after therapy initiation shows: Control: 2/10; AZD1775: 4/10; VAL083 7/10 and VAL-083+AZD1775: 11/11 mice alive. CONCLUSION: Our present study highlights that the combination of VAL-083 and AZD1775 might be a promising new therapeutic strategy for children with DIPG. Ongoing studies will continue to assess the in vivo activity as well explore the underlying mechanism of action of the combination strategy.

EXTH-10. A VERSATILE AND MODULAR TARGETED NANOPARTICLE PLATFORM FOR DELIVERY OF COMBINATION THERAPIES TO ADULT AND PEDIATRIC CNS TUMORS

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Effective treatment of CNS tumors is limited by the presence of the blood-brain barrier (BBB) and rapid resistance to single agent therapies. To address these issues, we developed nanoparticles (NPs) that can be functionalized with ligands, such as transferrin (Tf-NPs), for delivery across the BBB to deliver multiple cargo to CNS tumors. In vitro uptake studies in U87MG and GL261 GBM cell lines demonstrate increased intracellular uptake of Tf-NPs compared to non-functionalized NPs in a time-dependent manner. Using intravital imaging through a cranial window, we show the ability of Tf-NPs to traverse the intact BBB in non-tumor bearing mice as well as achieve direct and durable tumor binding in intracranial orthotopic models of U87MG and GL261 GBM. Treatment of tumor-bearing mice with Tf-NPs loaded with temozolomide (TMZ) and the bromodomain inhibitor JQ1 leads to superior therapeutic effects with increased DNA damage and apoptosis that correlates with a 1.5- to 2-fold decrease in tumor burden and corresponding increase in survival compared to mice treated with drugs packaged in non-functionalized NPs or mice treated with equivalent free-drug dosing. Immunocompetent mice treated with Tf-NP-loaded drugs also show relative protection from the effects of systemic drug toxicity due to TMZ and JQ1. Preliminary intravital imaging further shows the ability of our NPs to target pediatric tumors such as medulloblastoma, demonstrating the preclinical potential of this nanoscale platform to deliver novel combination therapies to adult and pediatric CNS tumors.

EXTH-11. GLIOBLASTOMA STEM CELL GROWTH DEPENDENCE ON NUTRIENTS: MORE THAN BASAL METABOLIC ACTIVITIES

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Deregulated uptake of glucose and amino acids is one of the hallmarks of cancer metabolism. Clinical trials and numerous drugs have been designed to target cancer cell metabolism, however, the effect on cell growth inhibition and how tumor cell metabolism couples with cell growth remain unclear. In this study, we report the glioblastoma stem cells (GSCs) growth dependence on nutrients (glucose, amino acid, and fatty acid) and oxygen. We show that the basal metabolic activities are unable to reflect the extent of proliferation inhibition by metabolic inhibitors. 2-DG (20mM) downregulated extracellular acidification rate (ECAR) more effectively in GSCs with higher glycolytic activity (Pearson r=0.71, p=0.08), the basal glycolytic activity, however, was not significantly correlated with glycolysis inhibitor (2-Deoxy-D-glucose, 2-DG) sensitivity (Pearson r=0.35, p=0.44), indicating basal glycolytic activity cannot predict GSC response to glycolysis inhibitor. Similarly, basal oxidative phosphorylation (OXPHOS) activity also failed to predict GSC responses to OXPHOS inhibition. Although GSCs showed the consistent susceptibility against oligomycin (complex V inhibitor) and hypoxia (Pearson r=0.61, p=0.15), there is no significant correlation between basal oxygen consumption rate (OCR) and oligomycin/hypoxia-induced proliferation inhibition. Thus, both basal glycolytic and basal OXPHOS activities were unable to explain their roles in GSC growth. Studies are underway to investigate the effect of fatty acid and amino acid on tumor growth. As drugs targeting cancer metabolic processes always show dose-limiting toxicity, our study will focus on identifying new "druggable" targets according to GSC nutrient dependences and developing specific therapeutic strategy for GBM populations with certain nutrient dependence.

EXTH-12. EFFECT OF THE PROTEIN ARGININE METHYLTRANSFERASE PRMT5 INHIBITION IN GLIOMA STEM-LIKE CELLS

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Protein arginine methyltransferases (PRMTs) have gained significant scientific attention due to their ability to regulate various biological functions including epigenetic regulation, cell differentiation, signal transduction and most importantly their dysregulation in human disease including cancer. Among various cancers, Glioblastoma is the most aggressive primary malignant brain tumor with few effective therapies. Currently, in glioblastoma therapy, the high expression level of Protein arginine methyltransferase 5 (PRMT5) has been reported as a therapeutic target which represses the transcription of a target gene via forming symmetric dimethylarginine (SDMA) residues. By using a panel of molecularly distinct glioma stem like cells (GSCs) we investigated the effect of PRMT5 inhibitor GSK591 on cell viability. Furthermore, genomic, proteomic (reverse protein lysate array, RPPA), methylation status and GSC subtype were correlated with GSK591 IC_{50} to find predictors of drug sensitivity. Western blotting data showed high expression of PRMT5 and multiple bands of SDMAs in most GSCs tested (n=31) irrespective of molecular subtype, indicating PRMT5 enzymatic activity in GSC cell lines. However, there is only a small subset of 4 GSC lines (13%) as showed complete inhibition of SDMA expression at low doses (< 1.5 μM) in dose- and time-dependent fashion after GSK591 treatment. The sensitivity of GSK591 correlated with low methylation of multiple genes pre-treatment, including MAGI2, EGR2, and DUSP16. In addition, up-regulated genes in sensitive GSCs correlated with proliferation signatures using gene set enrichment analyses (GSEA). Including this, we also found the upregulation of senescence marker along with activation of autophagy genes (Beclin-1 and Akt) in GSCs sensitive to GSK591. Tight regulation of Akt-mTOR pathway enables cancer cells to survive in harsh conditions via activation of autophagy signaling. Currently, we are investigating the role of PRMT5 in tumor growth through the concomitant regulation of autophagy signaling in GSCs.

EXTH-13. REDUCTION OF TUMOR BURDEN AND HEARING LOSS WITH A MULTIPLE RECEPTOR TYROSINE KINASE INHIBITOR BRIGATINIB IN A GENETICALLY ENGINEERED MOUSE MODEL OF NEUROFIBROMATOSIS TYPE 2

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Neurofibromatosis Type 2 (NF2) is an autosomal dominant genetic disorder caused by germline mutations in the tumor suppressor gene *NF2*, which encodes the protein Merlin. Patients with NF2 may develop bilateral vestibular schwannomas, which cause hearing impairment, and schwannomas in other regions of the nervous system. Due to the nature and location of the tumors, there is high morbidity and mortality associated with NF2, and no known effective chemotherapeutic intervention. A high-throughput *in vitro* cell viability screen revealed an Anaplastic Lymphoma Kinase (ALK) inhibitor as a top contender for selective inhibition of *Nf2*-deficient murine Schwann cells. Brigatinib (Alunbrig®) is a small molecule multiple receptor tyrosine kinase inhibitor that is FDA-approved for ALK-positive metastatic non-small cell lung cancer. Therefore, we hypothesized that brigatinib would prevent tumorigenesis in our genetically engineered mouse model of NF2 ($Nf2^{f/f}$; *Postn-Cre+*). 50mg/kg Brigatinib was administered to $Nf2^{f/f}$; *Postn-Cre+* mice by oral gavage daily for 12 weeks. Pharmacokinetic analyses paired with immunohistochemistry showed brigatinib is orally bioavailable, and treatment resulted in a therapeutically relevant concentration of brigatinib in the plasma (c_{max} = 4.8μM; t_{max} = 4hrs), which was sufficient to induce a biological response in the dorsal root ganglia (DRG) schwannomas. The volume of DRG in brigatinib-treated mice was 55% less than vehicle-treated mice. Additionally, brigatinib-treated mice did not have significant progression of hearing loss at the end of treatment, whereas vehicle-treated mice had a statistically significant 7.4dB increase in ABR threshold. Finally, histopathological assessment of DRG shows that brigatinib treatment prevented the progressive morphological disruption observed in the vehicle-treated mice. These data provide evidence that brigatinib targets may play a significant role in Merlin-deficient schwannomagenesis, and implicate brigatinib as a promising therapeutic intervention of NF2.

EXTH-14. REPURPOSING OF APPROVED DRUGS FOR THE TREATMENT OF GLIOMA STEM CELLS

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Glioblastoma (GBM) is the most common and malignant primary brain tumor. Glioma stem cells (GSCs), a subpopulation of GBM cells with stem cell properties, are responsible for the tumor initiation and recurrence, and therapeutic resistance. To develop novel therapeutic agents for sphere-forming patient derived GSCs, we utilized a new drug screening system and FDA approved drugs. The cell viability of the drugs on GSCs and normal human dermal fibroblast (nHDF) cells was determined by CellTiter-Glo 3D Cell Viability Assay or MTT assay. The relative cytotoxicity of drugs was compared with LC_{50}. We identified 8 drugs, including 4 that have not been studied for GSCs, which showed strong cytotoxic effect on sphere-forming GSCs but

were not toxic to nHDF cells in in vitro cell cultures. The LC_{50} of these identified drugs to GSCs is 10 fold to over 100 fold less than that for normal cells. Among the identified drugs, only one drug was not cytotoxic to GBM cells but toxic to GSCs. Based on the LC_{50} data and previous reports for the drugs on GSCs, we determined anti-tumor effects of two drugs in orthotopic brain tumor mouse models of human GBM cells. Animals treated with either drug (10 mg/kg once a week for 7 weeks or 30 mg/kg twice a week for 4 weeks) had significantly greater survival in Kaplan-Meier survival curve compared to vehicle treated control mice (p=0.03 or 0.05 in logrank test). Studies for structure-activity-relationship and mechanism of action are on-going. In summary, the present results provide compelling evidence that repurposing of approved drugs can be developed for GSCs and the study identified several selective cytotoxic drugs against sphere-forming GSCs. [This work was supported in part by the Tara Leah Witmer Endowment]

EXTH-15. TARGETING ANDROGEN SIGNALING IN GLIOBLASTOMA (GBM) USING SEVITERONEL (SEVI), A CYP17 LYASE AND ANDROGEN RECEPTOR (AR) INHIBITOR, ALONE AND IN COMBINATION WITH RADIATION (RT)

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The AR is a therapeutic target and a driver of growth and treatment resistance in prostate and breast cancer. Male sex is associated with increased GBM incidence and worsened prognosis but whether the AR is expressed or represents a therapeutic target in GBM is unknown. Using RNAseq and immunoblot, we found that GBM tumors, patient-derived xenografts and immortalized cell lines were frequently (i.e., >50%) AR (+) with expression levels that overlapped with prostate cancer. AR expression was 2-fold higher in GBM samples than normal brain tissue and was highest in the classical molecular subtype. Exogenous testosterone promoted the engraftment and growth of AR (+) T98G GBM xenografts in immunocompromised mice. SEVI, a blood-brain barrier permeable inhibitor of CYP17 lyase and the AR with clinical activity in prostate and breast cancer, inhibited the growth of AR (+) (T98G, LN18) but not AR(-) (8MGBA, AM38) GBM cell lines at achievable *in vivo* concentrations (GI_{50} = 3–4.0 uM) as measured by colony formation. Enzalutamide, an antiandrogen used clinically in prostate cancer but not GBM, also selectively inhibited colony formation in AR (+) GBM models, albeit at concentrations 3-6-fold higher than SEVI. SEVI selectively potentiated the action of RT in AR (+) GBM with enhancement ratios of 1.3–1.5 in clonogenic survival assays. Daily SEVI or RT slowed the growth of established androgen-driven T98G xenografts, which were further diminished by the combination of SEVI and RT. These results suggest that AR is frequently expressed in GBM and that androgen signaling promotes GBM growth in preclinical models. Inhibition of AR signaling alone or in combination with radiotherapy using clinical grade blood-brain barrier permeable antiandrogens may be a promising new treatment strategy for GBM.

EXTH-16. GOLPH3 SENSITIZES THE TUMOR SUPPRESSION EFFECT OF GEFITINIB ON GLIOMAS

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Golgi phosphoprotein 3 (GOLPH3) is associated with worse prognosis of gliomas, but its role and mechanism in glioma progression remain largely unknown. We previously reported that GOLPH3 promotes glioma progression by inhibiting EGFR endocytosis and degradation, leading to EGFR accumulation and PI3K-AKT pathway over-activation. In the current study, we examine whether GOLPH3 affects the response of glioma cells to gefitinib, an EGFR selective inhibitor. We found that both the immortalized and primary glioma cells with GOLPH3 over-expression hold higher EGFR protein levels on the cell membrane and exhibited higher sensitivity to gefitinib. In addition, primary glioma cells with higher GOLPH3 level exhibited stronger proliferation behavior. Importantly, GOLPH3 enhanced the anti-tumor effect of gefitinib in vivo. Consistently, after gefitinib treatment, tumors derived from GOLPH3 over-expression cells exhibited lower Ki67 positive and higher cleaved caspase-3 positive cells than control tumors. Taken together, our results demonstrate that GOLPH3 increases the sensitivity of glioma cells to gefitinib. Our study provides foundation for further exploring whether GOLPH3 high gliomas will more sensitive to gefitinib treatment in clinic and give ideas for developing new possible treatments for gliomas.

EXTH-17. SELECTIVE, NON-COMPETITIVE DRD2/3 ANTAGONISM BY IMIPRIDONE ONC206 IS EFFECTIVE IN TUMORS WITH DOPAMINE RECEPTOR DYSREGULATION

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Dopamine receptor D2 (DRD2) is a G protein-coupled receptor that is overexpressed in several cancers and its antagonism has been shown to cause anti-cancer effects. ONC201, an imipridone small molecule, is a DRD2/3 antagonist in Phase II advanced cancer clinical trials. We evaluated the binding target and anti-tumor activity of ONC206, a chemical derivative of ONC201. Experimental GPCR profiling with β-Arrestin recruitment assays revealed that ONC206 selectively antagonizes dopamine receptors DRD2 and DRD3. ONC206 exhibited a Ki of ~320nM for DRD2 with complete specificity across human GPCRs at doses that resulted in complete inhibition. Schild analyses of ONC206 in cAMP and β-Arrestin recruitment assays revealed hallmarks of non-competitive DRD2 antagonism, unlike antipsychotics that act as competitive and often non-selective antagonists. Shotgun mutagenesis across DRD2 identified 7 residues within and outside of the orthosteric binding site that are critical for ONC206-mediated antagonism. While 6 mutated residues were also critical for ONC201-mediated antagonism, the impact and magnitude of different mutants varied between the two compounds and one of the mutated residues was unique to ONC206 in an allosteric location. In vitro profiling of ONC206 in >1000 GDSC cancer cell lines revealed broad nanomolar efficacy (GI50 <78-889nM). TCGA and tissue microarrays analyses revealed that malignant DRD2 expression was highest in pheochromocytoma, high grade gliomas, neuroblastoma and medulloblastoma with nanomolar in vitro ONC206 sensitivity. A DRD2+/DRD5- RNA expression signature in the GDSC panel predicted significantly enhanced ONC206 sensitivity. ONC206 reduced the viability of normal human fibroblasts at micromolar doses (GI50 > 5μM), suggesting a wide therapeutic window. Robust inhibition of tumor growth was observed with weekly oral ONC206 (50mg/kg) in subcutaneous xenografts of cholangiocarcinoma, a dopamine-secreting bile duct tumor with DRD2 overexpression. Thus, imipridone ONC206 acts as a selective non-competitive DRD2/3 antagonist at nanomolar concentrations with efficacy in tumors with dopamine receptor pathway dysregulation.

EXTH-18. DEVELOPMENT OF ULTRASOUND-FACILITATED DRUG DELIVERY DEVICE FOR LOCAL DRUG INFUSION AGAINST BRAIN TUMORS

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OBJECT: Drug delivery against brain tumors is a challenge. Blood-brain barrier (BBB) and blood-tumor barrier always hinders the entry of therapeutic agents into brain tumors. On the other hand, local drug delivery also faces a major problem: limitations of distribution. Convection-enhanced delivery (CED) is a promising drug delivery method that deliver high-concentration drug directly into the targeted lesion beyond the BBB. We have been working to develop a novel treatment strategy with CED delivering chemotherapeutic agents directly into the tumor. Previously we developed an ultrasound facilitated drug delivery (UFD) system in order to achieve more robust drug distribution (J Neurosurg 124, 2016). In this study, we fabricated a second-generation device by analyzing optimal ultrasound generating condition and tested the intraparenchymal drug distribution using the device. METHODS: Resonance frequency of the device was determined by measuring the strength of sound field using hydrophone in water. Using the UFD system, applying resonance frequency, evans blue dye was infused into the striatum of Fisher344 rats. Immediately after the infusion, brains were harvested, quickly frozen, and sectioned to evaluate the distributions of the dye. RESULTS: The second-generation device successfully distributed the dye to the volume 1.5~2 times larger than simple CED with using half the driving voltage than first-generation device. Moreover, the distribution instability, which was a major problem with the first-generation device, was resolved achieving smaller standard deviation. Using multiple resonance frequencies of the device, we found the difference in drug distribution; suggesting the existence of optimal frequencies for brain interstitial drug delivery. CONCLUSIONS: The second generation UFD device successfully and stably achieved enlarged distribution in the brain parenchyma.

EXTH-19. WIRELESS INTRACRANIAL FLUORESCENCE MONITOR FOR DRUG DETECTION IN BRAIN TUMOR PATIENTS

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BACKGROUND: Currently, the brain penetration of drugs cannot be adequately evaluated in real-time, making chemotherapy selection for glioblastoma patients an unpredictable and inefficient process. Given recent progress in the microminiaturization of medical devices, our objective was to design and build the prototype of an implantable device allowing immediate quantification of fluorescent agents *in vivo*. METHODS: The Wireless Intracranial Fluorescence Monitor (WIFM) was assembled from commercially -purchased electrical and optical components, a custom-made printed circuit board (PCB), and 3D-printed couplers. Parts included a cyan LED, light-filtering lenses, optical fibers, a phototransistor, and a Bluetooth-enabled Arduino microcontroller. The filters prevented overlap of wavelengths from the LED and fluorophore emission spectra, ensuring isolated emission signals. Clinically, the WIFM would fit inside a standard Ommaya Reservoir, surgically implanted into brain parenchyma during craniotomy. The WIFM was tested *in vitro* in solutions of 0, 0.01, 0.05 and 0.10 mg/mL of fluorescein isothiocyanate (FITC), in PBS. RESULTS: FITC emission signals were converted into millivoltage readings, and transmitted to a laptop computer, allowing instantaneous wireless determinations. Voltage separations occurred 10–90 milliseconds after FITC excitation, were linearly related to FITC concentration, and allowed for FITC quantification by the device. Specifically, FITC concentrations of 0, 0.01, 0.05, and 0.10 mg/mL produced relative mV readings of 0, 30.6 ± 13.4, 99.9 ± 25.9 and 214.3 ± 21.0, respectively (n=15, $p<0.05$). CONCLUSIONS: While animal studies are needed in order to enable discrimination of interstitial fluorophore concentration, WIFM implantation offers the potential for real-time detection and quantification of the delivery of fluorescently-labeled drugs and/or nanoparticles to selected areas of the brain. Further iterations of the WIFM device should allow the administration of therapeutic agents (whether by systemic administration or convection-enhanced delvery) to be optimized, thus prolonging survival for patients with glioblastoma.

EXTH-20. HISTONE DEACETYLASE INHIBITOR ENHANCES ONCOLYTIC HERPES SIMPLEX VIRUS THERAPY FOR MALIGNANT MENINGIOMA

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Malignant (anaplastic) meningioma is a minor subset of meningioma that is lethal with no effective treatment options currently. Oncolytic herpes simplex virus (oHSV) is a powerful anti-cancer modality that induces both direct cell death and anti-tumor immunity. In neuro-oncology, oHSV has been extensively studied as a promising therapeutic for malignant glioma. We recently showed that oHSV G47delta demonstrated anti-tumor activity in a preclinical model of patient-derived malignant meningioma. However, direct injections of the oHSV in the orthotopic model in mice was not curative, revealing the need of improvement in the therapeutic approach. Epigenome modulator histone deacetylase inhibitor (HDACi) is an anti-cancer agent that has been shown to cooperate with oHSV. We here show that HDACi increases anti-cancer effects of oHSV in human malignant meningioma models, IOMM-Lee (NF2 wild-type) and CH-157 (NF2 mutant). Minimally toxic, sub-micromolar concentrations of pan-HDACi, Trichostatin A and Panobinostat, substantively increased the spread of oHSVs, G47delta and MG18L, within malignant meningioma cells in vitro. The robust spread of the virus resulted in enhanced oHSV-mediated killing of target cells when infected at low multiplicity of infection (MOI). Mechanistically, HDACi increased oHSV gene expression and replication via, at least partly, modulating interferon signaling pathway. In vivo studies to determine the beneficial impact of combining systemic HDACi with local oHSV administrations are currently underway. The combination of HDACi and oHSV may be effective at controlling malignant meningioma regardless of the tumor's NF2 status.

EXTH-21. REPURPOSING GLIOBLASTOMA EXOSOMES AS PERSONALIZED MULTI-ANTIGENIC ANTI-TUMOR VACCINE

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The composition of glial tumor derived exosomes (TEX) reflects plasma membrane and cytosol-derived proteins of the donor cell. Therefore, TEX form interesting candidates for anti-glioma skin dendritic cell targeted vaccination. Recent literature however, reports potent immune suppressive characteristics of TEX. TEX surface glycosylation may induce C-type lectin receptor triggering and/or cargo internalization thereby modifying the immunogenic/tolerogenic balance of the cellular immune response towards an immune suppressive phenotype. The aim of this study is to investigate whether we can repurpose TEX from immune suppressive vesicles towards immunogenic vehicles for multi-antigenic vaccination by *de novo* glycosylation of their surface. To this end, the surface glycosylation of TEX was carefully characterized by ELISA and electron microscopy. TEX uptake and downstream routing was assessed by imaging flow cytometry analysis. Our GBM-derived TEX were 50–200 nm in size and expressed high levels of CD63. The surface glycan profile of U251, U87, and GBM8-derived TEX was dominated by α-2,3 and α-2,6 sialic acid-capped *N*-glycans, high mannose glycans and truncated Tn-bearing *O*-glycans. These TEX appeared to have low affinity for DC-SIGN and MGL, but showed significant binding to Siglec-7 and Siglec-9. We developed a chemo-enzymatic glycan-modification protocol with the aim to replace Siglec ligands for DC-SIGN ligands in order to target them to skin resident dendritic cells for cross-presentation. Glycan-modified TEX, which had been introduced with a high affinity ligand for DC-SIGN, were efficiently taken up by human dendritic cells and fused with the endosomal pathway. In conclusion, repurposing glioma-derived TEX through *de novo* glycan-modification could provide a strategy for multi-antigenic anti-glioma vaccination.

EXTH-22. VERTEPORFIN TREATMENT INHIBITS GBM GROWTH AND MIGRATION AND INFORMS HIPPO/RTK CROSSTALK

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The diffusely infiltrative growth and spread in glioblastoma (GBM) impedes gross-total resection and chemoradiation. Tumor proliferation in GBM has been frequently related to aberrant activation of receptor tyrosine kinase (RTK) signaling. The Hippo pathway also regulates tissue growth and cell fate, and the dysregulation of its downstream effectors YAP1-TEAD has been implicated in tumor invasion, metastasis, and chemoresistance in RTK/RAS-driven carcinomas. The role of Hippo signaling and RTK crosstalk in GBM is still poorly understood. Recently, our lab defined a regulatory chromatin accessibility signature centered around the TEAD transcriptional family, which relates specifically to tumor migration in uncultured, patient-derived GBM stem cell populations, and we functionally validated TEAD1 as a driver of GBM migration, both in-vitro and in-vivo. Moreover, we found TEAD1 to be a direct transcriptional target of EGFR. To further characterize the effect of Hippo-TEAD on GBM migration and its interaction with EGFR/RTK signaling, we treated U87 and patient-derived GBM cells with Verteporfin (VP), an FDA-approved macular degeneration therapy, and a small-molecule inhibitor of the YAP/TEAD complex with proven anticancer efficacy. VP treatment inhibited not only GBM cell growth (IC_{50}=1.3μM) but also impaired tumor migration in three different in-vitro assays (live cell tracking, transwell invasion, and spheroid dispersion) in a dose-dependent manner (0.3μM/1.3μM/3μM). At the protein level, VP-treated cells showed dose-dependent downregulation of TEAD-target activity, including EGFR, as well as of downstream ERK phosphorylation. We then administered VP to mice with aggressive patient-derived orthotopic xenograft gliomas, through intraperitoneal injection for 8 days. Notably, VP penetrated into the brain parenchyma, and resulted in lower tumor burden without systemic toxicity (n=6). The inhibitory effect of Verteporfin on RTK signaling and GBM migration, and its brain penetrance at non-toxic levels in vivo, underscore potential future therapeutic value for this drug in GBM patients.

EXTH-23. A NOVEL NANOTECHNOLOGY-BASED PLATFORM IMPROVES LASER INTERSTITIAL THERMAL THERAPY FOR INTRACRANIAL TUMORS

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INTRODUCTION: Laser Interstitial Thermal Therapy (LITT) is an emerging clinical option for the thermal ablation of intracranial lesions. Current limitations for LITT, however, include the diameter of ablation (< 3cm per trajectory) and lack of inherent specificity for tumor margins. Gold nanoparticles act as "lightning rods" to expand laser treatment coverages. Here, we employ plasmon-activated gold nanostars (GNS) with selective tumor uptake to increase LITT coverage area and tumor specificity in a murine model, and also demonstrate their ability to expand coverage in a clinically-relevant sized model using brain phantoms. METHODS: CT2A murine glioma cells were implanted into the right cerebral hemisphere. GNS were administered intravenously. Selective GNS uptake in tumors was demonstrated by micro-PET-CT. Microlaser fibers (700um) were used to deliver laser light to tumors. Brain phantoms were composed of agarose gel with embedded 5x5x5 cm^3 agarose "tumors" with or without interspersed GNS. A diffuse-tip probe was used to deliver laser light to phantom tumors in the intraoperative MRI suite. MRI thermometry measured temperature

change in phantoms. RESULTS: Systemically administered GNS were selectively taken up by tumor compared to surrounding brain. Murine gliomas treated with GNS + laser showed an expanded tumor-conforming zone of cytotoxic edema on small animal MRI compared to tumors treated with laser alone. In phantoms containing GNS, the kill zone (43°C for 10 min) extended to 3.8cm in diameter, compared to 2.0cm in phantoms without GNS. Additionally, the phantoms containing GNS heated faster, reached higher temperatures, and displayed a more homogeneous zone of treatment. CONCLUSION: We demonstrate the capacity to use nanotechnology to overcome the size and specificity limitations for LITT, both in an *in vivo* murine intracranial glioma model, as well as in a more relevantly sized brain phantom model. Clinical trials in canine glioma are planned to enable an IDE application.

EXTH-24. EXPOSURE TO TUMOR TREATING FIELDS INHIBITS DNA REPAIR, INDUCES REPLICATION STRESS AND RENDERS TUMOR CELLS SENSITIVE TO AGENTS THAT IMPINGE UPON THESE PATHWAYS

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Tumor treating fields (TTFields) are low-intensity, intermediate frequency, alternating electric fields applied non-invasively across a tumor. Optune (NovoCure), a TTFields delivery device, has been approved for recurrent and newly diagnosed glioblastoma and clinical trials are ongoing for other cancers. To further understand the molecular mechanisms underlying TTFields sensitivity beyond the anti-mitotic effects already described, we monitored temporal gene expression changes in 5 non-small cell lung cancer (NSCLC) cell lines whose response to TTFields is variable. We found the expression of the BRCA1 DNA damage repair pathway, as well as other DNA repair/checkpoint pathways were significantly downregulated (P<0.05). Quantitative measurement of DNA double strand break repair revealed that TTFields treatment slowed the repair of ionizing radiation (IR)-induced DNA damage and interestingly, TTFields alone increased the number of γH2AX foci and the incidence of chromatid aberrations. Furthermore, as a function of TTFields exposure time, the length of newly replicated DNA was shorter and R-loop formation increased, suggesting that TTFields induce replication stress. Based on these newly identified mechanisms of TTFields action in DNA damage and replication stress pathways we hypothesized that by applying TTFields first, a conditional vulnerability environment would develop rendering cells more susceptible to agents such as radiation, cisplatin, or PARP inhibition. In agreement with our hypothesis, NSCLC cell susceptibility to radiation increased when cells were exposed to TTFields prior to IR treatment compared to IR treatment followed by TTFields. Furthermore, we found that the effect of TTFields exposure concomitant with the PARP inhibitor Olaparib followed by radiation was synergistic compared to radiation or Olaparib alone or in combination, although the degree of sensitization and synergy varied across the different cell lines. Based upon these results we suggest the clinical use of TTFields as neoadjuvant therapy prior to radiation treatment and either prior to or concomitant with chemotherapy agents.

EXTH-25. DIANHYDROGALACTITOL (VAL-083) REDUCES GLIOBLASTOMA TUMOR GROWTH IN VIVO, UPON BEVACIZUMAB-INDUCED HYPOXIA

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Standard-of-care for glioblastoma (GBM) includes surgery, radiation and temozolomide. Nearly all tumors recur and 5-year survival is less than 3%. Unmethylated promoter status for O^6-methylguanine-DNA-methyltransferase (MGMT) is a validated biomarker for temozolomide-resistance. Second-line treatment with bevacizumab has not only failed to improve survival, but has also been shown to induce intratumor hypoxia, which is implicated in increased chemoresistance. VAL-083 is a bi-functional DNA-targeting agent that readily crosses the blood-brain barrier. VAL-083 targets N^7-guanine, causing DNA double-strand breaks and cancer cell-death in GBM cancer stem cells (CSCs) and non-CSCs, independent of MGMT. We have previously shown that bevacizumab treatment upregulates expression of glucose transporters GLUT-1/GLUT-3 on GBM cells. We hypothesized that, based on its unique monosaccharide backbone structure, VAL-083 may benefit from bevacizumab-induced GLUT transporter upregulation leading to enhanced VAL-083 uptake and anti-tumor activity. Methods: To investigate the *in vivo* anti-tumor effect of VAL-083+bevacizumab, we used a orthotopic patient-derived xenograft GBM model. All mice carried *MGMT*-unmethylated, temozolomide-resistant recurrent T16 GBM tumors as detected by MRI 35 days post-implantation. Mice were grouped into control, bevacizumab, VAL-083, and VAL-083+bevacizmab. Tumor progression was measured by MRI on days 49 and 56, and tumor growth rate was calculated for the entire study (day 35 vs. 56) and for the last 7 days (day 49 vs. 56). Results: Tumors were significantly smaller in VAL-083-treated mice both compared to control (-83%, p<0.001) and compared to bevacizumab-treated (-75%, p<0.001) mice. Additionally, analysis of tumor growth in-time showed significantly reduced tumor growth rate for VAL-083+bevacizumab compared to VAL-083 alone (p<0.01). Conclusions: These results show strong *in vivo* anti-tumor efficacy of VAL-083 against *MGMT*-unmethylated, temozolomide-resistant recurrent GBM. This effect was further augmented in combination with bevacizumab, providing rationale of clinical investigation in combination with bevacizumab in the treatment of GBM.

EXTH-26. MOLECULAR DETERMINANT OF CLINICAL RESPONSE TO ONC201, AN INHIBITOR OF DOPAMINE RECEPTOR 2 (DRD2) SIGNALING, IN GLIOBLASTOMA PATIENT

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INTRODUCTION: ONC201 is a highly selective antagonist of dopamine receptor D2 (DRD2) that is in phase II clinical trial as treatment for recurrent glioblastoma. Here we examine the molecular determinants of therapeutic response that are associated with long-term survivors. METHODS: Immunohistochemical (IHC) analysis/pathway-based transcriptomal analysis of clinical glioblastoma specimens, *in vitro*- and *in vivo*-studies of dopamine antagonist inhibition, analysis of glioblastoma specimens in patients treated with the dopamine receptor antagonist, ONC201. RESULTS: In IHC of clinical glioblastoma samples, we found that the expression of dopamine receptor 2 (DRD2) coincides with EGFR over-expression in mesenchymal and classical glioblastomas. In contrast, DRD2 over-expression was found in proneural and neural glioblastomas in the absence EGFR over-expression. These patterns of expression were recapitulated in The Cancer Genome Atlas (TCGA) as well patient derived glioblastoma (PDX) lines. *In vitro* and *in vivo*, mesenchymal glioblastoma PDX lines with high expression of EGFR and DRD2 were refractory to DRD2 or EGFR inhibition; simultaneous EGFR and DRD2 inhibition were required to arrest the growth of these mesenchymal glioblastomas. In contrast, proneural glioblastoma lines with high DRD2 expression (and low EGFR expression) were exquisitely sensitive to dopamine antagonists *in vitro* and *in vivo* (in subcutaneous and intracranial models). Importantly, proneural glioblastoma lines with high DRD2 expression secrete high levels of dopamine, creating autocrine mitogenic signaling that drive glioblastoma growth. In clinical samples derived from a Phase 2 clinical trial where recurrent glioblastoma patients were treated with the DRD2 antagonist, ONC201, long-terms survival (>2 years survival after glioblastoma recurrence) was observed only in patients afflicted with glioblastomas characterized by low EGFR and high DRD2 expression. CONCLUSION: Our data suggest that DRD2 signaling is essential in glioblastomas that are not mitogenically driven by EGFR signaling. These tumors appear exquisitely sensitive to ONC201 mediated DRD2 inhibition.

EXTH-27. INFLAMMATORY REPROGRAMMING OF GLIOMAS USING DELTA-24-RGDOX AND IMMUNOMETABOLIC ADJUVANTS

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The immunosupressive microenvironment of glioblastoma (GB) is responsible for the resistance to therapies and its dismal prognosis. Several lines of evidence have linked brain tumor metabolism and immunoresistance with the presence of active metabolic pathways. In this regard, activation of indolamine-2,3-dioxygenase (IDO) in regulatory T-cells favors tumor immunosuppression. We have observed that the implantation of tumors in mice generates an immunosuppressive environment characterized by over-representation of CD4+ regulatory T-cells and MDSCs. Treatment with the oncolytic adenovirus, Delta-24-RGD, has been shown to induce complete responses in a subset of GB patients by reprogramming the immune response. Moreover, the anti-tumor cytotoxic properties of T-cells can be enhanced by the addition of immune agonists, such as OX40L, a T-cell activator. We hypothesized that the combination of IDO inhibition (1MT or Indoximod) and Delta-24-RGD armed with the OX40 ligand (Delta-24-RGDOX) synergizes and have a therapeutic effect in GB. In this study, orthotopic GB were implanted in immunocompetent mouse and treated with the combination of D24-RGDOX and 1MT. Delta-24-RGDOX treatment resulted in a significant survival benefit compared to the control groups

(mean survival, 46.5 vs. 38.5 days, *P*=0.02). Interestingly, the combination treatment of Delta-24-RGDOX and the IDO inhibitor induces complete tumor regression and the production of a higher percentage of long-term survivors compared to single therapy with Delta-24-RGDOX (mean survival, 46.5 vs. 108.5 days, *P*=0.03). Moreover, when the long-term glioma survivors were re-challenged with similar glioma cells, we observed 100% survival (*P*=0.002), indicating the establishment of immune memory by the combination therapy. Furthermore, functional studies showed a significant increase in anti-tumor activity of splenocytes from GB bearing treated mice that were treated with the combined therapy D24-RGDOX and Indoximod (*P*=0.009). These data support the use of immunometabolic adjuvants in combination with virotherapy as a potential treatment of patients with GB.

EXTH-28. ENHANCING SELUMETINIB-MEDIATED KILLING OF SHH MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common primary malignant pediatric brain cancer and consists of at least 5 distinct molecular subgroups. Of these subgroups, Sonic Hedgehog (SHH) MB exhibits extensive cellular heterogeneity, increased SHH signaling, and has an intermediate prognosis. Treatment strategies employing SHH pathway inhibitors showed initial promise, however, acquired drug resistance has led to therapy failure in clinical trials. Consequently, improved chemotherapeutic strategies designed to better treat SHH MB must be identified. A major contributing factor to cellular heterogeneity, and thus drug resistance and disease progression, are rare putative brain tumor-propagating cells (TPC). As a result, identifying robust TPC biomarkers can reveal novel subgroup-specific therapies capable of targeting these elusive cell types. We recently identified the neurotrophin receptor (CD271) within the SHH MB subgroup as a promising biomarker delineating TPC populations and associated with elevated MEK/ERK signaling. Moreover, we showed that selumetinib, a MEK inhibitor, reduced CD271 levels, decreased TPC growth, and extended survival in mouse models. While promising, the mice in our *in vivo* studies ultimately succumb to disease progression; thus, an enhanced selumetinib-based chemotherapeutic strategy is merited. In this study, we employed RNA-sequencing to identify 543 genes differentially expressed in selumetinib resistant xenografts relative to controls. Further, gene set enrichment analysis revealed significant changes in specific signaling pathways predicted to underlie selumetinib resistance and contribute to SHH MB progression. Accordingly, to ultimately enhance survival, we employ our high-content tumorsphere assay to systematically evaluate candidate blood brain penetrant inhibitors that are highly predicted to elicit therapeutic synergy in combination with selumetinib *in vivo*. In addition, we will test selumetinib in combination with current SHH MB standard of care chemotherapeutics to characterize survival. Our studies will reveal mechanisms of SHH MB drug resistance, and identify novel drug combinations that may ultimately enhance MB patients' survival and quality of life.

EXTH-29. MULTI-RECEPTOR TARGETING IN GBM

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Glioblastoma is a heterogeneous tumor for which all single-targeted agents failed. Being that a cytotoxic cocktail targeting IL-13RA2 and EphA2 receptors is effective in the treatment of spontaneous gliomas in dogs, we have been further pursuing molecular resection by targeting four tumor-specific/associated receptors at once: IL-13RA2, EphA2, EphA3 and EphB2. These receptors are over-expressed in most GBM tumor compartments responsible for tumor progression and recurrence. To this end, we have engineered a protein based on an immunoglobulin scaffold with a ligand targeting the three Eph receptors, ephrinA5 (eA5), at the N-terminal end and a ligand targeting IL-13RA2, IL-13.E13K, at the C-terminal end (QUAD). However, the protein produced in insect cells had frequently two bands on SDS-PAGE. We have therefore decided to change the order of the ligands and placed the ligand targeting IL-13RA2 at the N-terminal end of the protein and the other ligand and the C-terminus. We also started using High-Five cells for codon and yield optimization. The newer construct is produced as a single protein band on the SDS-PAGE and Western blot and can be purified to homogeneity. It bound all four receptors effectively in ELISA while the affinity toward the EphA3 was even better than in the original multivalent compound. We also obtained higher yields of the protein. To make our vector even more versatile, we have added a C-terminal cysteine providing a free thiol group for chemical conjugation with drugs. The QUAD-Cys was also obtained as a single band protein homodimer and the multi-targeted protein bound effectively to all targets of interest. Thus, we have optimized the production of a functional multivalent targeted vector protein for making drug conjugates. QUAD and QUAD-Cys based conjugates will be tested for their utility in glioma treatment using relevant clinical models like spontaneous brain tumors in dogs.

EXTH-30. IN SITU AUTOVACCINATION MEDIATED BY ONCOLYTIC ADENOVIRUS DELTA-24-RGDOX INDUCES EFFICACIOUS IMMUNITY AGAINST METASTATIC MELANOMA

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We have reported that Delta-24-RGDOX, an oncolytic adenovirus expressing the immune co-stimulator OX40 ligand (OX40L), induces efficacious anti-glioma immunity in syngeneic intracranial glioma models of immune competent mice. Here we sought to determine if the local injection of the virus triggers a systemic immune response against metastatic melanomas. We found that Delta-24-RGDOX expressed OX40L and induced cell lysis in both human and mouse melanoma cells. To study the abscopal effect of Delta-24-RGDOX against metastatic melanomas, we established s.c./s.c. and s.c./i.c. melanoma models with B16-Red-FLuc cells in C57BL/6 mice. In the s.c./s.c. model, we found that three doses of intratumoral injection of the virus significantly inhibited the growth of the injected and the untreated distant tumors, resulting in prolonged survival of the mice (70% long-term survival; $P = 0.001$). The surviving mice were resistant to a rechallenging with the implantation of similar tumor cells, but not when lung cancer cells were implanted, strongly suggesting the development of specific tumor immune memory. Using flow cytometry analysis, we found that Delta-24-RGDOX treatment resulted in increased frequency of CD3+ T lymphocytes, CD3+CD4+ helper T cells, CD3+CD8+ cytotoxic T cells and effector T cells, and a decrease in the amount of T regulatory cells. In addition, we observed an increment of effector CD4+ and CD8+ T cells frequency with PD-1 expression on spleen and blood cells, indicating a systemic activation of anti-tumor circulatory cells. In the s.c./i.c. model, viral injection into the s.c. tumor induced anti-melanoma activity in the brain, resulting in growth inhibition of both tumors and prolonged survival of the animals. In agreement with these data, viral injection in the s.c. tumor increased the frequency of the T effector cells in the brain tumor. In summary, local treatment of melanoma with Delta-24-RGDOX results in suppression of distant tumors including brain melanoma.

EXTH-31. TARGETING THE FORMIN-DIRECTED CYTOSKELETON WITH SMALL MOLECULE AGONISTS BLOCKS GLIOBLASTOMA PATIENT-DERIVED NEUROSPHERE INVASION

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Glioblastoma multiforme (GBM) is the most common primary malignant brain tumor in adults and aggressive treatment only extends survival by months. While metastasis outside the CNS is rare, GBM is highly invasive. Failure of current GBM therapies to target invasive cells partly explains why these treatments confer only minimal survival advantages: invasive tumors lack easily-defined margins, making complete surgical resection impossible, and invasive GBM cells are inherently more chemo- and radioresistant. Therefore, anti-invasive therapies may effectively sensitize GBM cells to conventional therapies and improve survival. Anti-invasive treatments are thus greatly needed, and cellular mechanisms governing GBM invasion represent understudied therapeutic targets. Much work has centered upon how Rho GTPases mediate GBM invasion, yet the roles of downstream Rho effector proteins are poorly understood and represent potential novel therapeutic targets. A role for the mammalian Diaphanous (mDia)-related formin family of Rho GTPase effector proteins has emerged in metastatic disease. mDias are nanomachines generating linear actin filaments to drive protrusive cytoskeletal structures underlying tumor cell invasion. Using novel small molecule mDia agonists (IMMs, orn intramimics) that induce endogenous mDia functional activities, including F-actin polymerization, we demonstrated roles for mDia in driving polarized GBM cell migration. mDia agonism halted GBM spheroid invasion in three-dimensional (3D) *in vitro* and *ex vivo* rat brain slice models. Here, we evaluate if GBM patient cell lines are sensitive to formin agonism to halt invasion. Four patient-derived GBM cell lines were isolated as single cell suspensions, and spontaneously formed non-adherent neurospheres. Neurospheres were embedded in 3D-matrices and allowed to invade +/- IMMs. IMMs dramatically inhibited GBM patient neurosphere invasion, significantly impacting both distance single cells migrated from neurosphere edges and lengths of actin-enriched cellular extensions into matrices. Thus, mDia agonism effectively disrupted multiple aspects of patient-derived GBM neurosphere invasion *in vitro*, warranting further investigation in patient-derived xenografts.

EXTH-32. TRANSGENIC EXPRESSION OF IL15 IMPROVES THE EFFICACY OF CAR T CELLS IN AN IMMUNE COMPETENT GLIOBLASTOMA MODEL

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BACKGROUND: Malignant gliomas (MG) are the most common and difficult-to-treat adult brain tumors. The outstanding efficacy of chimeric antigen receptor (CAR)-modified T cells against hematological malignancies gives hope that they can be programmed to target and eradicate solid tumors like MG. We recently demonstrated that murine CAR.CD28.ζ T cells targeting the tumor-associated antigen interleukin-13 receptor alpha 2 (IL13Rα2) have antitumor activity in two syngeneic models of MG in comparison to control animals. However, the majority of tumors recurred. The goal of this project was now to explore if transgenic expression of IL15 improves their anti-glioma activity. METHODS/RESULTS: We generated a retroviral vector encoding murine IL13Rα2-CAR.CD28.ζ, a 2A peptide, and secretable IL15 (IL13Rα2-CAR.IL15), and a control vector encoding a non-functional CAR (IL13Rα2-CAR.Δ), a 2A peptide, and IL15 (IL13Rα2-CAR.Δ.IL15). Murine CAR T cells were generated by activating and transducing murine CD3$^+$ T cells with retroviral particles. The effector function of IL13Rα2-CAR, IL13Rα2-CAR.Δ, IL13Rα2-CAR.IL15, and CAR.Δ.IL15 were compared *in vitro* and *in vivo*. Only CAR T cells expressing functional CARs killed IL13Rα2+ GL261 (GL261-IL13Rα2) glioma cells. In addition, only IL13Rα2-CAR.IL15 and IL13Rα2-CAR.Δ.IL15 T cells secreted IL15 as judged by ELISA. *In vivo*, GL261-IL13Rα2 glioma-bearing mice treated with a single i.t. dose of IL13Rα2-CAR.IL15 T cells had a significant improved survival in comparison to mice treated with IL13Rα2-CAR T cells. This therapeutic benefit was antigen-specific since CAR.Δ.IL15 T-cells did not improve animals' survival. CONCLUSIONS: Our data demonstrates that IL13Rα2-CAR.IL15 T cells have greater antitumor activity *in vivo* than IL13Rα2-CAR T cells in an immune competent glioma model. Thus, 2nd genetic modifications have the potential to improve current CAR T-cell therapy approaches for MG.

EXTH-33. COMPREHENSIVE TARGETING OF NOVEL MASTER REGULATORS OF CANCER STEM CELLS TO TREAT GLIOBLASTOMA – IN VIVO STUDY

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Glioblastoma (GBM) is the most prevalent and aggressive brain cancer in adults. GBM is enriched with glioma stemlike cells (GSCs) that contribute to tumor initiation and treatment resistance and thus a natural focus for therapeutic development. However, targeting GSCs has been a challenge because of the dearth of knowledge of master regulators specific to GSCs and not to normal brain cells. Therefore systematic elucidation of the GSC specific core regulatory program will improve our understanding of GSC biology and provide an opportunity to develop novel GSC specific therapy with maximal efficacy and minimal toxicity. To accomplish these objectives, we have recently developed two innovative computational platforms, GeneRep and nSCORE and experimentally validated them in several published datasets. With this platforms, a cluster of 6 interrelated master subnetworks emerged, which were functionally grouped into the stemness and cancer pathways, some of which are known Olig and Myc factors. By enforcing the expression of this cluster in normal Human Astrocytes, we were able to reprogram these normal cells into GSC-like cells as measured by the neurosphere formation and in vivo tumorigenesis assays, confirming the contribution of this cluster for the GSC development. Furthermore, this cluster was present and functioned cooperatively to maintain GSCs in patient derived GSC lines. Functionally blockage individually or two of them by shRNA significantly reduces neurosphere formation and promotes cell death. Intracranial Injection of those gene-modified-GSCs into NSG mouse, dramatically double the mouse survival time. Most promising, preliminary data shows that *in vivo* tumor growth could be inhibited by either modulation of gene expression or via cancer vaccine. Our data establish a novel set of genes controlling GSCs survival and provide a compelling rationale for its therapeutic targeting to reduce tumor growth.

EXTH-34. G-QUADRUPLEX DNA DRIVES GENOMIC INSTABILITY AND REPRESENTS A TARGETABLE MOLECULAR ABNORMALITY IN ATRX-DEFICIENT MALIGNANT GLIOMA

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Mutational inactivation of *ATRX* (a-thalassemia mental retardation X-linked) represents a defining molecular alteration in large subsets of malignant glioma. *ATRX* encodes a chromatin binding protein widely implicated in epigenetic regulation and remodeling. However, multiple studies have also associated its loss in cancer with replication stress, DNA damage, and copy number alterations (CNAs). The mechanisms by which ATRX deficiency drives this global genomic instability remain unclear. Here we report that *ATRX* inactivation in isogenic glioma model systems induces replication stress and DNA damage by promoting the formation of deleterious G-quadruplex (G4) secondary structure in DNA. Moreover, these effects are associated with the acquisition of disease-relevant CNAs over time. Prior work has linked G4s with genomic instability as well as CNAs in cancer. We then demonstrate, both *in vitro* and *in vivo*, that chemical G4 stabilization with CX-3543 (Quarfloxin) selectively enhances cell death in ATRX deficient isogenic cell lines as well as *ATRX*-mutant primary glioma stem cells derived from patients. Finally, we show that G4 stabilization synergizes with other DNA-damaging therapies, including ionizing radiation, in the ATRX-deficient context. Our findings clarify distinct mechanisms by which DNA secondary structure influences ATRX-deficient glioma pathogenesis and indicate that G4-stabilization may represent and attractive therapeutic strategy for the selective targeting of *ATRX*-mutant cancers. Opportunities for the development of radiosensitization approaches based on G4-stabilization are particularly intriguing, as ionizing radiation remains among the most effective non-surgical treatments for malignant glioma.

EXTH-35. IN VIVO ^{1}H MRS DETECTS REDUCED 2HG PRODUCTION IN IDH1 MUTANT GLIOMAS TREATED WITH A DUAL PI3K/MTOR INHIBITOR

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70–90% of low-grade gliomas and secondary glioblastomas are characterized by mutations in isocitrate dehydrogenase (IDH1mut). Mutant IDH produces the oncometabolite 2-hydroxyglutarate (2HG), which drives tumorigenesis. Inhibitors of phosphoinositide-3-kinase (PI3K) pathway are currently under clinical investigation for IDH1mut gliomas with positive outcome results, but early response imaging biomarkers are missing. The goal of this study was to identify potential magnetic resonance (MR) detectable biomarkers of IDH1mut glioma response to the dual PI3K/mammalian target of rapamycin (mTOR) inhibitor XL765. We used two genetically-engineered IDH1mut models: a U87 glioblastoma-based model (U87IDHmut) and an immortalized normal human astrocyte-based model (NHAIDHmut), as well as a patient derived IDH1mut model (BT142). The reduction of PI3K/mTOR pathway signaling was validated with western blots of p4E-BP1 for all models. We then used MR spectroscopy (MRS) to investigate the impact of treatment on cell metabolism. Using ^{1}H-MRS, we observed that XL765 induced a significant ~70% and ~50% reduction in steady-state levels of 2HG and glutamate in U87IDHmut while in NHAIDHmut the drop was ~90% and ~70% for 2HG and glutamate respectively. In the case of BT142 there was a ~35% drop in glutamate levels while 2HG was MRS undetectable in both groups. The translatability of our findings was evaluated in mice bearing orthotropic U87IDHmut xerographs treated with XL765 orally twice a day. XL765-treatment of mice led to an apparent slower tumor growth, which was associated with significantly increased animal survival. Importantly, in vivo ^{1}H-MRS spectroscopy showed a significant reduction in 2HG levels. The results were verified by ^{1}H-MRS of tumor extracts. Collectively, our results indicate that treatment with XL765 is associated with response in IDH1mut models. To our knowledge, this is the first time in vivo ^{1}H-MRS detected reduction in 2HG in gliomas undergoing treatment with a non-IDH1mut-specific inhibitor. Thus, 2HG could potentially be used as a response biomarker.

EXTH-36. BIFUNCTIONAL RNA NANOPARTICLES INDUCE ANTITUMOR IMMUNE RESPONSES AND ALLOW MRI-BASED DETECTION OF DENDRITIC CELL MIGRATION AS A BIOMARKER OF ANTITUMOR IMMUNE RESPONSE

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BACKGROUND: Cancer vaccines are a promising approach to personalized cancer immunotherapy, but the lack of meaningful biomarkers of patient response to treatment limits their development. We recently reported in a randomized and blinded pilot clinical trial that RNA-pulsed dendritic cells (DCs) combined with tetanus-diphtheria (Td) booster vaccine prolong progression-free and overall survival in patients with glioblastoma (Mitchell et al, *Nature* 2015). Furthermore, we demonstrated that DC migration to lymph nodes assessed by SPECT/CT imaging strongly correlates with clinical outcomes. While this finding may provide a novel imaging biomarker for response to DC vaccines, the complexity and regulatory requirements of nuclear medicine-based imaging of radiolabeled cells limits widespread utilization of this technique. We have therefore developed bi-functional RNA-loaded magnetic nanoparticles to load DCs with RNA-encoded antigens, enhance DC migration to lymph nodes, and track migration *in vivo* using an MRI-based imaging modality. METHODS: Immune-stimulatorycationic liposomes with iron oxide nanoparticle cores were complexed with mRNA. The resulting iron oxide-loaded RNA-NPs (IO-RNA-NPs) were used to transfect DCs *ex vivo* in the presence of a magnetic field. IO-RNA-NP-loaded DCs were then injected intradermally into tumor-bearing C57Bl6 mice and tracked noninvasively with T2-weighted 11T MRI. RESULTS: The presence of iron oxide in RNA-NPs did not significantly alter particle characteristics. Additionally, inclusion of iron oxide within RNA-NPs enabled magnetically enhanced transfection through application of external magnetic fields. Compared to RNA electroporation, IO-RNA-NP loading enhanced production of inflammatory cytokines and DC migration to lymph nodes. IO-RNA-NPs also produced a reduction in T2-weighted MRI intensity and an increase in MRI-detected lymph node size that correlated directly with the number of iron oxide loaded DCs in treated lymph nodes, inhibition of tumor growth, and survival in murine tumor models. CONCLUSION: This data suggests that IO-RNA-NPs enhance DC activation and establish MRI-detected dendritic cell migration as a biomarker of antitumor vaccine response.

EXTH-38. A NEW COMPUTATIONAL METHOD FOR COMPREHENSIVE ESTIMATION OF ANTI TUMOR EFFICACY OF TUMOR TREATING FIELDS (TTFIELDS). ACCOUNTING FOR FIELD INTENSITY, EXPOSURE TIME AND UNWANTED SPATIAL FIELD CORRELATION

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INTRODUCTION: The non-invasive glioblastoma treatment, tumor treating fields (TTFields, Optune®), uses alternating electrical fields (200 kHz) to inhibit cancer cell division. TTFields are induced by two sequentially active pairs of transducer arrays placed on the patient's scalp. Finite element (FE) methods are used to estimate the field intensity of TTFields. To date, calculations have focused entirely on field intensity, neglecting field angle and exposure time that also influence efficacy. Based on principal component analysis, this study derives a novel approach to quantify mean intensity of TTFields together with unwanted effects from spatial field correlation, indexed as fractional anisotropy (FA). MATERIALS AND METHODS: Distributions of the two sequential TTFields (50% duty cycle, 2 s total duration) were calculated using FE methods on a realistic glioblastoma head-model (calculated based on images before and after resection) using four different array layouts. In each element of the model, the combined fields were decomposed into principal components. For each element, intensity, and directional correlation of the average field were calculated as the square root of the electrical energy (Frobenius norm) and the FA, respectively. RESULTS: Significant unwanted FA was observed within several regions of the brain, particularly at resection borders. These effects may potentially reduce therapeutic efficacy of TTFields. FA varied between different layouts, suggesting a different array performance than predicted from conventional intensity calculations. Resection of a tumor increased FA and removed differences between layouts. CONCLUSIONS: This study questions the rationale for the general use of macroscopically orthogonal layouts to reduce field correlation. The results indicate that in some cases, arrays should be placed differently to maximize pathology coverage and field intensity. We present a new and comprehensive framework for TTFields characterization that potentially could improve treatment planning, technology development, and accurate prognostication models. Further studies are required to validate the method.

EXTH-39. DETECTION, MOLECULAR PROFILING AND CULTURE OF CSF-CTCs IN LEPTOMENINGEAL DISEASE (LMDz) IN MELANOMA TO IMPROVE DIAGNOSIS AND TREATMENT STRATEGIES

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BACKGROUND: Approximately 5% of melanoma-associated brain metastasis also develops universally fatal LMDz. The aim of this study was to improve diagnosis and personalized treatment for melanoma-LMDz by enumerating CTCs from CSF. METHODS: CSF-CTCs were enriched and detected by Veridex CellSearch® System and the circulating melanoma cell kit. Cell-free DNA and cell-associated DNA were extracted, sequenced and profiled. Expanded *ex-vivo* CSF-CTCs and murine BRAF V600E mutant SM1 cells were labeled with viral fluorophore-NanoLuc BRET and injected into the cisterna magna of immunocomprised mice. These cells were also tested for drug sensitivity *in-vitro*. RESULTS: CSF-CTCs: 12 patients with definite LMDz and 8 melanoma patients without LMDz were studied. All but 1 LMDz patients (92%) had CSF-CTCs (23 CTCs/ml to 3055 CTCs/ml CSF). In contrast, only 3/8 (37%) melanoma patients without LMDz had CSF-CTCs detected, with significantly lower CTC counts per ml CSF (0.13 CTCs/ml to 0.6 CTCs/ml CSF). CSF-CTCs Profile: LMDz patients showed GNAQ Q209P mutation (uveal melanoma), NRAS Q61R mutation (nasal melanoma) and also BRAF V600E mutation. *Ex vivo* culture of CSF-CTCs and *in-vivo injections*: We successfully demonstrated *ex-vivo* expansion of isolated CSF-CTCs (~25% of samples). Drug treatment revealed Ceritinib could kill BRAF-inhibitor resistant melanoma-CTCs. Mice injected with SM1-GFP-NanoLuc exhibited tumor growth in ~1.5 weeks. Metastasis was detected in the brain and spinal cord regions. CONCLUSIONS: Though current patient size is small, this is the first report of the successful culture and drug testing of CSF-CTCs from patients with LMDz. Single cell analysis and *in-vivo* testing in progress.

EXTH-40. OPTIMIZING ARRAY LAYOUTS FOR GLIOBLASTOMA THERAPY WITH TUMOR TREATING FIELDS (TTFIELDS) –USE OF OBLIQUE ARRAY LAYOUTS SURPASS DEFAULT LEFT-RIGHT/ANTERIOR-POSTERIOR POSITIONS IN A COMPUTER SIMULATION MODEL

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INTRODUCTION: Tumor treating fields (TTFields, Optune®) is an effective treatment for glioblastoma. The antimitotic effects of TTFields are induced by low-intensity, intermediate frequency (200 kHz) alternating electric fields, delivered through two pairs of transducer arrays placed on the patient's scalp. The present study aimed to identify optimal array positions that induced the highest electric field in the tumor by analyzing systematic variations in array layouts. MATERIALS AND METHODS: TTFields distribution was computed using finite element methods with a realistic computational head model. A standard anterior-posterior (AP) layout was rotated in 15-degree intervals in the same plane around a central cranio-caudal axis of the head to investigate thirteen array positions. During subdivision, tumors were placed at nineteen different frontoparietal positions in the array rotation plane. RESULTS: TTFields distribution was affected by different array layouts. Two array layouts were identified to be suitable for most tumors. These identified positions led to TTFields intensities that were approximately 30–40% higher in the tumors than in standard AP and left/right (LR) layouts. The two optimal layouts were oriented at 90-degree intervals to each other. Subsequent analysis of combining two array pairs revealed a single optimum layout. For each tumor position, only one optimum layout combination was identified, which was usually oriented in 15–45 degree angles relative to the sagittal plane. In each case, an oblique layout that was orientated at 45 degrees to the sagittal plane was effective for most tumor localizations and superior to the default AP/LR layout combination. Determining factors for layout optimization were high field intensities at transducers located on the edges of the arrays, with high fields close to the peripheral transducers. CONCLUSIONS: The present study provides guiding principles for optimal TTFields layout design and planning. Individual patient-specific models should be used to determine TTFields distributions more accurately.

EXTH-41. EFFECTS OF TUMOR TREATING FIELDS (TTFIELDS) ON BLOOD BRAIN BARRIER (BBB) PERMEABILITY

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BACKGROUND: Drug application for the treatment of malignant brain tumors, in particular glioblastoma multiforme (GBM), can be impeded by the

blood–brain barrier (BBB). Recently, low intensity, intermediate frequency alternating electric fields called Tumor Treating Fields (TTFields) have been established as novel adjuvant treatment modality for GBM. Here, the effect of TTFields on BBB permeability is inspected. MATERIAL AND METHODS: Immortalized murine brain capillary endothelial cells (cerebEND) that were grown on coverslips and transwell inserts, were treated with TTFields at a frequency of 100–300 kHz for up to 72 h. Immunofluorescence staining of the tight-junction proteins Claudin 5 and ZO-1 was utilized to analyze cell morphology. BBB integrity was determined using transendothelial resistance (TEER) and BBB permeability was checked with flow cytometry analysis applying fluorescein isothiocyanate (FITC). In rats, Evans Blue (EB) was utilized to quantify the increase in vessel permeability during TTFields application to the brain (100 kHz, 72 h). RESULTS: TTFields application disturbs the BBB by delocalization of tight junction proteins from the cell boundaries to the cytoplasm with most dramatic effects at 100 kHz. The BBB integrity was significantly reduced by 65% and the BBB permeability for 4 kDa large molecules was significantly increased upon TTFields application. The cell morphology started to recover 48 h and was completed 96 h after treatment end pointing to a reversibility of the TTFields-effect on the BBB. TTFields application to the rat head significantly increased the average accumulation of EB in the brain. CONCLUSION: As TTFields at a frequency of 100 kHz may potentially permeabilize the BBB, they could be utilized to deliver drugs generally unable to cross the BBB to the central nervous system. The presented *in vitro* and *in vivo* data may lead to a phase I clinical trial and clinical application in the future.

EXTH-42. CANSCRIPT MAY PREDICT A SUBSET OF GLIOBLASTOMAS THAT RESPOND TO SP1 BLOCKERS

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INTRODUCTION: Perturbation of SP1 has long been implicated in the pathogenesis and drug resistance in Glioblastomas. Multiple mechanisms including upregulation of BACH 1 by antagonizing the function of P53 and hypomethylating MGMT have been described. In addition, the increased expression of genes involved in promoting cancer stem cells may orchestrate this resistance. METHODS: Biopsied GBM samples (n=25) were evaluated for response to SP1 inhibitor using CANscript, a clinically relevant *ex vivo* platform. CANscript system incorporates a personalized tumor microenvironment approach for treatment evaluation and yields a high degree of predictive correlation to patient clinical outcomes (Majumder B *et al. Nature Communications*, 2015). RESULTS: Out of the 25 samples evaluated 10 samples were predicted to respond to the SP1 inhibitor with a CANscript score of more than 25. This finding is in line with the percentage of SP1 sensitivity seen in other cancers. While the SP1 level was not consistently elevated in this subset, stem cell markers like NANOG, BMI1 and CD133 were found to be differentially expressed in selected cases. An association of resistance and hypomethylation of MGMT was also found with SP1 pertubation was found. CONCLUSION: A significant subset of glioblastomas may display dependency on SP1driven pathways and inhibitors that target this pathway have the potential to positively impact treatment of this subset of tumors. REFERENCE: Predicting clinical response to anticancer drugs using an ex vivo platform that captures tumour heterogeneity. Majumder B, et.al

EXTH-43. EFFECTIVE TREATMENT OF CANINE SPONTANEOUS GLIOMAS WITH A CYTOTOXIC COCKTAIL TARGETING IL-13RA2 AND EphA2 RECEPTORS

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Malignant gliomas are incurable tumors and canine spontaneous gliomas are the closest model to human disease. In our Phase I canine clinical trial we are delivering a cocktail targeting IL-13RA2 and EphA2 receptors containing modified bacterial cytotoxins using convection enhanced delivery (CED). Dogs with forebrain gliomas demonstrating IL-13RA2 and EphA2 positivity were included. Using a 3 + 3 dose-escalation design, cohorts were administered 0.05, 0.1, 0.2, 0.4, or 0.8 μg of each cytotoxin/ml of infusate. CED was planned using a shape-fitting algorithm. Cytotoxins were co-administered with gadolinium through reflux preventing catheters to allow intraoperative MRI visualization of infusions. Clinical examinations, adverse events (AE), and volumetric tumor responses were evaluated on days 14, 28, 42, 84, 180, 270, and 365 following treatment. Grades 3, 4, or 5 AE developing within 28 days of infusion were considered dose-limiting toxicities (DLT). Sixteen CED infusions were performed in fourteen dogs with gliomas (astrocytomas, n=5; oligodendrogliomas, n=7; mixed gliomas, n=2). The median target volume was 5.47 cm^3 (0.69 to 11.4 cm^3). The median target coverage was 77% (38–96%). MRI monitoring facilitated correction of technical complications observed in 5/16 infusions that allowed continued target coverage. The 3-, 6-, 9-, and 12-month progression-free survival rates were 57% (8/14), 38% (5/13), 23% (3/13), and 0% (0/12), respectively. Major tumor responses (52–95% volumetric decreases) associated with clinical improvement have been observed in 5/8 dogs with ≥3 months of follow-up. Tumor necrosis in infused regions was evident in post-mortem examinations in 5 dogs dying of progressive disease. DLT have not been observed. The use of therapeutic planning, intraoperative MRI, and reflux-preventing catheters allowed for safe and effective CED of IL-13RA2 and EphA2 targeted cytotoxins. This study provides evidence of efficacy of the cocktail and warrants further clinical evaluation in humans.

EXTH-44. TARGETING GLIOMA STEM CELLS WITH CAR-T IMMUNOTHERAPY IN XENOGRAFT ANIMAL MODELS

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Recurrent Glioblastoma (GBM) tumors often arise from glioma-initiating cells or glioma stem cells (GSCs). GSCs are usually resistant to the standard therapeutic regiment of radiation and chemotherapy and hence have been an attractive target for the development of immunotherapeutic strategies to treating recurrent GBMs. Chimeric antigen receptor expressing engineered T (CAR-T) cells are redirected to detect and destroy rare tumor cells that express specific tumor antigens. CAR-T cells have been successful in treating hematological cancers, and moderately effective in remission of primary GBM tumors, but not yet been successful in the immunotherapeutic targeting of GSCs or recurrent GBMs. In this study, we have treated two patient-derived GSCs and tumor cell line derived neurospheres, as well as GSC/neurosphere-derived secondary tumor cells with CAR-T cells that specifically target IL13Rα2 tumor antigens on GBMs. These GSCs and neurospheres were resistant to radiation and adjuvant chemotherapy but were successful in activating antigen-specific CAR-T cells- as observed by increased proliferation as well as secretion of cytokines. In vitro co-culture of CAR-T cells with GSCs, neurospheres, and secondary tumors resulted in the effective cytotoxic killing of tumor cells. In experimental animals bearing xenograft implantation of GSCs or redefined secondary tumor cells, tumors went into remission when treated with CAR-T cells, in comparison to treatment with T cells that did not express specific CAR molecule. Together, we conclude that CAR-T cell immunotherapy can be an effective approach to targeting GSCs and treating secondary or recurrent GBM tumors.

EXTH-45. THE REPURPOSED CUSP9 REGIMEN EXERTS POTENT ANTI-NEOPLASTIC ACTIVITY AGAINST GLIOBLASTOMA CELLS IN VITRO

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OBJECTIVE: Drug repurposing is a strategy to safely accelerate the clinical application of therapeutics that provide anti-cancer activity. CUSP9 represents such a therapeutic regime which includes nine repurposed drugs along with low-dose temozolomide. In this work, we examined the biological activity of the CUSP9 regimen in an *in vitro* setting of glioblastoma. METHODS: MTT and soft-agar assays were used to examine cellular proliferation. Staining for Annexin V/PI and Western blotting were used to examine pro-apoptotic effects. A spheroid assay was performed to assess effects of the treatments on three-dimensional growth. Time-lapse microscopy, radius and transwell assays were performed to examine anti-migratory effects. RESULTS: Treatment with CUSP9 exerted profound anti-proliferative activity and inhibited anchorage-independent growth among a broad panel of established, primary cultured and glioma stem-like cells. These effects were accompanied by enhanced apoptosis as indicated by an increased fraction of Annexin V-positive cells and enhanced caspase-3 cleavage. Moreover, CUSP9 lead to significantly decreased 3-dimensional tumor formation. In addition, non-directed and directed movement of glioblastoma cells were significantly impaired following CUSP9 treatment. CONCLUSION: These data suggest that CUSP9 has potent anti-cancer activity *in vitro* which is currently further explored by a clinical trial being conducted at our institution (NCT02770378).

EXTH-46. A COMBINATORY IMMUNOTHERAPY AGAINST BRAIN TUMOUR: BLOOD DENDRITIC CELL BASED VACCINE THERAPY WITH CHECKPOINT INHIBITOR(S)

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Cancer vaccines represent a novel and promising approach for aggressive neoplasms such as glioblastoma multiforme (GBM) where other treatment modalities have not been effective. In this regard, cancer vaccines have been developed which exploit the key central immunoregulatory dendritic cell (DC) to maximize vaccine efficacy. Such "DC vaccine" strategies are currently being evaluated clinically and form the basis of a number of commercial initiatives. However, current monocyte derived DCs (Mo-DCs) based vaccines have shown limited efficacy, possibly due to insufficient antigen presentation capability as well as inability to migrate toward lymph nodes. In this regard, we have developed a novel antibody against the CMRF-56 antigen which preferentially selects for distinct blood derived dendritic cell (BDC) subsets including myeloid CD1c+ and the highly efficient cross-presenting CD141+ BDC subsets. Unlike commonly used Mo-DC vaccines, which require artificial induction of differentiation/activation to load tumour specific antigens, CMRF-56 antibody-based selected BDCs showed highly activated/matured status upon selection. BDCs also showed the better migratory capability in response to the lymph nodes-migratory signal (CCR7/CCL21). Most importantly, antigen processing and presentation capability and glioma specific cytotoxicity mediated by BDC activated T cells were superior to those of Mo-DCs, implying highly efficient immunotherapeutic efficacy against GBM. In addition, we will discuss a potential boosting combinatory immunotherapeutic approach against GBM; BDC based vaccine in combination with checkpoint inhibitor, anti-PD1. In this research, we will demonstrate a therapeutic potential of a novel immune-combinatory personalized medicine against GBM.

EXTH-47. DEVELOPMENT OF A NOVEL FLUORESCENT VIRUS-LIKE-PARTICLES AS RNA INTERFERENCE AND TUMOR SUPPRESSOR GENE DELIVERY TOOL FOR BRAIN TUMOR TREATMENT

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Efficient delivery is a key issue in translating RNA interference (RNAi) technology into a feasible therapy. The efficiency of carrier systems used for this technology is commonly tested by co-transfection, i.e. simultaneous transfection with an exogenous gene and with the siRNA. Here, we describe a fluorescence virus-like particles (fVLPs) platform with self RNAi production and packaging to inhibit gene expression and overexpress tumor suppressor proteins simultaneously for efficient cancer therapy. We have designed an RNAi scaffold (let-7g, c-Met, Wnt-2 siRNA) and co-expressed with Qβ coat protein and mCherry fluorescent protein simultaneously through a two-plasmid system, and self-assembly produced in *E. coli*. The polylysine (PLL) would be incorporated to the exterior surface of fVLPs to enhance cell uptake and adsorb plasmid DNA on the surface. The PLL-mediated fVLPs have been assessed by cell examination. In our study, The fVLPs revealed no cytotoxicity and could be internalized inside cells. Our data showed that the PLL-mediated fVLPs could effectively knockdown let-7g gene to inhibit the cancer growth and also suggested that this platform is amenable to efficient packaging of functional RNAi and, most recently, simultaneous packaging with plasmid DNA. Then we used brain tumor model animals for testing the tumor suppress efficiency *in vivo*. The tumor growth rate is significantly reduced in siRNA loaded VLP-administrated animals and longer survival periods is observed. Also, there is no significant alterations to the blood biochemical values of the VLP-administrated animals. We believe this multifunctional PLL-mediated fVLPs platform has potential to overcome impediments as mentioned earlier and well suited for RNAi-based therapeutic tools.

EXTH-48. ORAL GALLIUM MALTOLATE IMPAIRS TUMOR GROWTH AND EXTENDS DISEASE-SPECIFIC SURVIVAL IN A XENOGRAFT MODEL OF RECURRENT GBM

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BACKGROUND: Recurrent glioblastoma (rGBM) is a distinctly different disease condition than *de novo* GBM, characterized by chemo- and radioresistance. Consequently, treatment options remain limited. Given our recently published promising results using the novel iron mimetic, gallium maltolate (GaM), in a rat xenograft of *de novo* GBM, we set out to investigate the effects of oral GaM in an *in vivo* model of rGBM. METHODS: Irradiated adult human GBM cells were stereotactically implanted into the right striatum of 12 male athymic rats. Following confirmation of *in vivo* tumor growth by advanced MRI at 9.4T on day 14 post-implantation, animals received GaM (50 mg/kg/day, n=5) in an oral preparation for voluntary ingestion. Two animals received daily oral GaM continuously throughout the study period, while three underwent a 2-week on, 1-week off treatment cycle. Tumor growth was monitored weekly by MRI, and lesion volume and associated parameter maps were determined using enhancing tumor ROIs. RESULTS: Mean compliance with voluntary ingestion of GaM was 97% (49 mg/kg/day). Complete longitudinal MRI data was available for 5 GaM rats and 5 controls. The mean weekly tumor growth rates of enhancing lesions were 78% and 156% in GaM-treated and control animals, respectively (p=0.006). Median disease-specific survival was 28 days in controls and 49 days in GaM-treated animals, with 3/5 treated animals still alive (p=0.004). Preliminary histological findings in GaM-treated tumor tissue indicate treatment effect (swollen cells, degeneration and/or granulation of cytoplasm) and apoptotic cells throughout the lesions, compared to pseudopalisading necrosis and standard tumor cell size in control tissues. No adverse physiological effects due to treatment were noted. CONCLUSION: We presented compelling evidence that oral GaM significantly impairs tumor growth and extends disease-specific survival in a xenograft model of rGBM. We propose that GaM shows great promise as a new interventional strategy for GBM therapy.

EXTH-49. NOVEL AD-REIC VECTOR WITH THE SUPER GENE EXPRESSION (SGE) SYSTEM (AD-SGE-REIC) AS A PROMISING THERAPEUTIC AGENT FOR MALIGNANT GLIOMA

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INTRODUCTION: Reduced expression in immortalized cells/Dickkopf-3 (REIC/Dkk-3) is a tumor suppressor and therapeutic gene in many human cancers. We have investigated the anti-glioma effect of the adenovirus vector carrying REIC/Dkk-3 (Ad-CAG-REIC). Recently an Ad-REIC vector with the super gene expression (SGE) system (Ad-SGE-REIC) has been developed for higher protein expression and therapeutic effects than the conventional adenoviral vector (Ad-CAG-REIC). In this study, we evaluated the anti-glioma effect of the Ad-SGE-REIC against malignant glioma. MATERIALS AND METHODS: Transcriptome analysis of the differential (exome sequencing-derived) expression levels of REIC was conducted based on using The Cancer Genome Atlas (TCGA) GBM patient dataset (n = 349) using the Project Betastasis web platform (http://www.betastasis.com/glioma/tcga_gbm/). To evaluate the anti-glioma effect of the Ad-SGE-REIC, we conducted a cytotoxicity assay to assess treatments with Ad-SGE-REIC, Ad-CAG-REIC, or Ad-LacZ (control) using malignant glioma cells (U87ΔEGFR or GL261) and normal human astrocytes (NHAs). Seven days after implantation of glioma cells into the brain of mice, Ad-SGE-REIC, Ad-CAG-REIC, or Ad-LacZ (control) was injected stereotactically at the tumor inoculation site. The survival of mice in each group was analyzed by the Kaplan–Meier method. RESULTS: Analysis of the TCGA GBM patient dataset revealed that differential expression levels of REIC were lower in all subgroups, including Proneural, Neural, Classical, and Mesenchymal groups, than in the control group. In the cytotoxicity assay, after treatment with Ad-SGE-REIC, the number of malignant glioma cells attached to the bottom of culture wells was significantly reduced in a time-dependent manner. The survival time of the mice treated with Ad-SGE-REIC was significantly longer than those treated with Ad-LacZ or Ad-CAG-REIC. CONCLUSIONS: We demonstrated the anti-glioma effect of Ad-SGE-REIC. We are now planning an investigator-initiated clinical trial (phase I/IIa) of Ad-SGE-REIC for the treatment of recurrent malignant glioma.

EXTH-50. NOVEL IODINE NANOPARTICLES (INPs) FOR RADIATION ENHANCEMENT OF BRAIN TUMORS

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Nanoprobes Inc. in collaboration with the Smilowitz lab pioneered the use of non-toxic 15nm gold nanoparticles (AuNPs) to enhance radiation therapy (RT) of advanced murine EMT-6 mammary tumors growing subcutaneously (Hainfeld et al., 2004) and advanced gliomas growing in the brains of mice (Hainfeld et al., 2013). Because of skin discoloration and clearance concerns, second generation INPs were developed by Nanoprobes, Inc. These 20nm non-toxic particles (blood half-life 40 hrs) passively and selectively leak into brain tumors after IV injection. Tumor accumulations, determined by MIcroCT, were seen throughout the tumors, were macroscopically heterogeneous and achieved clinically relevant levels. Liver INP clearance was 50% over 6-months. Fluorescence microscopy

showed INPs localized to the region of the brain containing the advanced glioma. Confocal microscopy (63X) revealed INPs largely associated with the tumor endothelium and the surface of tumor cells with some evidence of tumor cell internalization. Using advanced U87 tumors growing in the brains of athymic nude mice, IV injection of 3.5 and 7g/kg INP twenty-four hours prior to single-dose 15Gy RT (RT100KeV X-Ray source) provided median life extensions of ~2 and 2.4-fold, respectively. Tumors followed by bioluminescence showed an initial slowing of the tumors followed by a slower rate of increase after the administration of INPs. Median life extension was also seen in the context of fractionated RT (2x10Gy). Importantly, INP-enhanced RT synergistically increased the efficacy of DOXIL chemotherapy administered at 15mg/kg given in six IV doses over two weeks. Canine safety studies and clinical trials in dogs with gliomas are warranted prior to human translation.

EXTH-51. C18-CERAMIDE ANALOGUE DRUG OVERCOMES RESISTANCE TO TEMOZOLOMIDE IN GLIOBLASTOMA

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Glioblastoma (GB) is a WHO Grade IV brain tumor with very limited therapeutic option currently available. The DNA-alkylating agent temozolomide (TMZ) is currently the most effective chemotoxic drug for GB therapy but efficacy tends to be short-lasting with tumors eventually becoming resistant to treatment. Developing novel agents is therefore of paramount importance. Ceramide synthase 1 (CERS1) is the most highly expressed CERS in the central nervous system, and ceramide with an 18-carbon–containing fatty acid chain (C18-ceramide) plays important roles in signaling and sphingolipid development. However, the roles of CERS1 and C18-ceramide in glioma are largely unknown. Our results demonstrated C18 ceramide is down regulated in 70% of human GB tumor tissues as compared to non-tumor tissues, indicating that down-regulation of C18-ceramide synthesis might have a role glioma-genesis. These roles were examined by reconstitution of C18-ceramide in GB cells via addition of exogenous C18-ceramide analogue drug, or overexpression of CERS1, which has been shown to specifically induce the generation of C18-ceramide. Our results demonstrated that C18-ceramide reconstitution using pharmacologic or molecular tools induced cell death in human GB cells that exhibited resistance to TMZ, but had no effect on cell death induction in normal astrocytes. Thus, these data suggest that reconstitution of C18-ceramide signaling induces cell death in TMZ resistant GB.

EXTH-52. USE OF A PHOSPHOLIPID BINDING MARCKS MIMETIC FOR TARGETED KILLING OF GLIOBLASTOMA CELLS

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Glioblastoma (GBM) like most cancers harbors frequent mutations in phospholipid signaling that contributes to many of the hallmarks of cancer, including immune suppression from the externalization of phosphatidylserine (PS) in viable cells, to pro-growth, survival and invasive signaling resulting from mutations in phosphoinositide (PI) metabolizing enzymes like phosphoinositide 3-kinase p110α (PIK3CA). We evaluated the therapeutic potential of using a PS and phosphatidylinositol 4,5-bisphosphate (PIP2) binding peptide derived from Myristoylated Alanine-Rich C-Kinase Substrate (MARCKS) effector domain (ED) to suppress GBM growth. The conjugation of a cell penetrating trans-activator of transcription (TAT) sequence to MARCKS ED (TAT-ED) was found to have potent dose-dependent cytotoxicity to GBM patient-derived xenografts (PDX) at low micromolar doses, 20 times greater than the unconjugated ED peptide while remaining nontoxic to normal human astrocytes. A Cy7 labeled TAT-ED showed substantial punctate accumulations at the plasma membrane of GBM but only rarely on NHA's. Quantification of the TAT-ED uptake using the Xcyto10 image cytometer showed TAT-ED accumulates to greater levels in the cytoplasm and nucleus of GBM, poorly penetrates into the nucleus of NHA's, and revealed high levels of TAT-ED was associated with DNA hypoploidy. TAT-ED was equally cytotoxic to PDX neurospheres and adherent cells and found to trigger a rapid and sustained rise in intracellular calcium, with the appearance of a unique annexin V positive bleb, preceding a caspase-independent cell death and simultaneous annexin V positivity and membrane permeability. *In vivo* bio distribution studies revealed TAT-ED crosses the blood-brain barrier concentrating in the periventricular region of tumor naive mice or in intracranial tumors. Kinomic and mRNA pathway analysis suggests TAT-ED activates PKC, NRD2 and MAPK pathway, while inhibiting ephrin receptors and SRC family kinases. Overall, this study finds TAT-ED to have both selective and potent therapeutic effects against GBM.

EXTH-53. IN VIVO QUANTITATIVE ANALYSIS OF ONCOLYTIC VIRUS-TUMOR KINETICS

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Oncolytic virus (OV) immunotherapy is becoming a clinically feasible therapy for cancer, with one FDA-approved product and many more in the clinical trial pipeline. Yet, there are still gaps in mechanistic knowledge related to how much oncolysis and OV replication is needed to achieve efficacious cytotoxic T cell responses. We have developed a series of "tools" to understand the kinetics of OV replication and correlate with antitumor efficacy. These consist of an oncolytic HSV that expresses two luciferase isoforms (Rluc and Fluc) activated at immediate early or late stages of viral replication cycle and U87dEGFR cells expressing a secreted luciferase (Cluc). Cluc levels in serum significantly correlated with tumor volumes measured by MRI (r = 0.9653). U87dEGFR-Cluc tumors in the brains of athymic mice were treated with this oncolytic HSV. Temporal analyses of the OV and tumor luciferases clustered mice into those that responded to therapy as assayed by stable MRIs or did not (MRIs showing increasing tumor volumes). While there was no significant difference in maximum tumor infection level by OVs between responders vs. non-responders (p = 0.17), responders showed significantly higher levels of replication (p = 0.036), followed by significantly faster decrease in both OV and tumor-expressed luciferases (p < 0.0001). Non-responders exhibited enlarging areas of tumor necrosis measured by T2 weighted MRI (increased from 10.3 to 17.4mm^3 on day12-16 compared to a decrease from 10.8 to 4.7mm^3 in responders) which correlated with the signal intensity of OV-expressed Rluc and Fluc (r = 0.6807 and 0.5526, respectively). Histologically, responders showed an even biodistribution of OV in tumors with densely recruited Iba-1 positive cells while non-responders had uneven distribution sometimes with tumor hemorrhages (3/6 in non-responders, 0/2 in responders). These analyses can help us better understand the results of OV therapy from clinical trials.

EXTH-54. Bcl-2/Bcl-xL INHIBITION SYNERGISTICALLY ENHANCES THE ANTI-NEOPLASTIC ACTIVITY OF CUSP9 AGAINST GLIOBLASTOMA CELLS IN VITRO

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OBJECTIVE: Repurposing represents a promising approach to safely accelerate the clinical application of therapeutics with anti-cancer activity. In this study, we examined whether inhibition of the anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-xL enhances the biological effects of the repurposed CUSP9 regimen in an *in vitro* setting of glioblastoma. METHODS: We applied MTT assays to assess cellular proliferation. Annexin V/PI and TMRE staining were used to examine apoptosis. Western blotting, RT-PCR and specific knockdown experiments using siRNA were employed to examine molecular mechanisms of action. RESULTS: Bcl-2/Bcl-xL inhibition by the BH3 mimetic ABT263, yielded synergistic anti-proliferative effects across a wide panel of established and primary cultured glioblastoma cells when combined with CUSP9 which had been reduced to only one tenth of its original concentration (CUSP9 1/10). The combination treatment also led to enhanced apoptosis with loss of mitochondrial membrane potential and activation of caspases. On the molecular level, CUSP9 1/10 counteracted ABT263-mediated upregulation of Mcl-1. Silencing of Mcl-1 enhanced ABT263-mediated apoptosis, indicating that Mcl-1 is crucial for the induction of cell death conveyed by the combination treatment. Levels of Mcl-1 mRNA were not decreased following combination therapy, and co-treatment with cycloheximide showed reduced protein stability, pointing towards a post-translational mechanism of action. CONCLUSION: These data suggest that Bcl-2/Bcl-xL inhibition enhances the susceptibility of glioblastoma cells towards CUSP9, allowing dramatic dose reduction and potentially decreased toxicity when applied clinically. A clinical trial involving the original CUSP doses (CUSP9v3) is currently ongoing in our institution (NCT02770378). The Bcl-2/Bcl-xL inhibitor ABT263 is in clinical trials and might represent a valuable adjunct to the original CUSP.

EXTH-55. CONCOMITANT INHIBITION OF RAC1 AND Bcl-2/Bcl-xL INTERFERES WITH THE Mcl-1/Usp9X AXIS AND YIELDS SYNERGISTIC ANTI-GLIOMA ACTIVITY

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The purpose of this study was to examine whether inhibition of RAC1 would enhance the pro-apoptotic reprogramming of glioblastoma's cellular circuitry following selective Bcl-2/Bcl-xL inhibition by BH-3 mimetics. Preclinical drug testing and molecular profiling was performed in different glioblastoma model systems including established, primary and glioma stem-like cells. Our data show that combined inhibition of RAC1 and Bcl-2/Bcl-xL resulted in synergistic anti-proliferative and pro-apoptotic effects in a panel of different glioblastoma cells. RAC1 inhibition lead to decreased expression of the deubiquitinase Usp9X and depletion of endogenous Mcl-1 through a post-translational mechanism. The combination treatment diminished the expression of the anti-apoptotic Bcl-2 family proteins Bcl-2 and Bcl-xL. In addition, the migratory activity of glioblastoma cells was significantly inhibited by the combination treatment. Lastly, tumor formation on the chorion allantoic membrane of chicken embryos was significantly impaired by simultaneous inhibition of RAC1 and Bcl-2/Bcl-xL. In conclusion, our data suggest that inhibition of RAC1 strongly enhances the pro-apoptotic shift in glioblastoma cells induced by BH-3-mimetics via counteracting mechanisms of resistance such as upregulation of Mcl-1. From a mechanistic point of view, RAC1 inhibition affects protein stability of Mcl-1 which is likely to be subsequent to decreased expression of Usp9X. Overall, the promising biological anti-cancer activity of this multi-targeting strategy warrants further *in vivo* testing.

EXTH-56. CLINICALLY DELIVERABLE DEEP BRAIN STIMULATOR GENERATED ELECTRICAL FIELDS HAVE PROFOUND EFFECTS ON GLIOBLASTOMA MULTIFORME CELL LINES

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INTRODUCTION: Phase III trials of the Optune system which utilises Tumour Treating Fields (TTFields) have shown positive results in both primary, and recurrent adult Glioblastoma multiforme (GBM) patients. These results have given strength to the feasibility of electromagnetic fields as a therapy for brain tumour patients. Here we present the repurposing deep brain stimulation (DBS) electrodes as a novel delivery system of therapeutic electric fields to GBM cell lines. METHODS: Medtronic DBS electrodes were inserted into cell culture flasks and delivered clinically relevant electric fields over a range of frequencies and intensities to our panel of GBM cell lines. The effects of DBS treatment on cell viability, cell cycling, long-term effects of treatment, as well as genome-wide expression via microarray were analysed. GBM cells were also treated in a combinational fashion with mitotic inhibitors to enhance efficacy. RESULTS: DBS electric fields negatively affect cell proliferation and viability of our commercial and primary GBM cell lines over a range of clinically relevant DBS settings. The magnitude of these effects were dependent upon frequency, intensity and the number of DBS leads. Cells treated with DBS were re-seeded and growth rates were compared to non-treated cells. The treated cells experienced up to 50% slower growth rates following treatment. Cell cycle analysis revealed that DBS treated cells have significant levels of G_0 phase accumulation relative to control flasks. The effects of electrotreatment on gene expression will be discussed. Efficacy of DBS may be increased with the addition of paclitaxel and mebendazole. CONCLUSIONS: DBS treatment has demonstrated efficacy against our array of GBM cell lines. The treatments offers an alternative electric fields treatment to TTFields, which has different mechanisms of action at the cellular level and this is reflected in our data.

EXTH-57. PLASMA AND CEREBROSPINAL FLUID PHARMACOKINETICS OF THE PROCASPASE-ACTIVATING COMPOUND, PAC-1, FOLLOWING ORAL ADMINISTRATION IN A NON-HUMAN PRIMATE MODEL

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BACKGROUND: PAC-1 is a novel compound which induces apoptosis via activation of the procaspase-3 pathway in cancer cells. Human clinical trials are ongoing with PAC-1 as a single agent (late-stage cancers) and in combination therapies (malignant glioma), in addition to studies in pet canines (glioma). This study evaluated the CSF penetration of PAC-1 via plasma and CSF pharmacokinetics in a nonhuman primate CSF Ventricular Reservoir or Lumbar Port model that allows serial CSF collection. METHODS: 4 male rhesus macaques received 15 mg/kg (Human Equivalent Dose of 558 mg/m^2/day) of PAC-1 in 6 studies with two formulations; liquid (n=1) or pill (n=5). 1 animal received both formulations. Paired plasma and CSF (lumbar n=2 and ventricular n=4) samples were collected for 0–72 hours. PAC-1 was quantified by LC-MC/MS. The lower limit of quantitation for plasma was 10.0 ng/ml and CSF 0.2 ng/ml. PK parameters were calculated via noncompartmental methods. RESULTS: 5 studies were evaluable (n=1 liquid and n=4 pill). Plasma-Quanitifible (n=5). Liquid Formulation (n=1): $AUC_{0-\infty}$ 5299.91 hr*ng/ml, Half-Life 4.99 hr, and Clearance 57.43 L/hr/m^2. Pill Formulation PK range (n=4): $AUC_{0-\infty}$ 1527.7–8036.08 hr*ng/ml, Half-Life 10.2–31.5 hr, and Clearance 37.65–199.49 L/hr/m^2. CSF-Quanitifible (n=3; 2 lumbar & 1 ventricular) or undectable (n=2 ventricular). Liquid Formulation (lumbar n=1): $AUC_{0-\infty}$ 50.90 hr*ng/ml, Half-Life 4.59 hr, and Clearance 5979.56 L/hr/m^2 Pill Formulation PK range (lumbar n=1 and ventricular n=1): $AUC_{0-\infty}$ 7.53–31.25 hr*ng/ml, Half-Life 22.35–28.88 hr, and Clearance 9955.96–40178.44 L/hr/m^2. CSF: Plasma Ratio formulation comparison: 0.96% liquid and 0.46% pill. CONCLUSIONS: In this animal model, the CSF penetration of PAC-1 is low and formulation-dependent, with CSF: Plasma ratio 2 fold greater with liquid verses pill formulation. The CSF clearance is rapid. FUTURE STUDIES: 10.08 and 12.1 mg/kg dosages (Human Equivalent Dose 375 and 450, mg/m^2/day, respectively) are currently undergoing evaluation in this NHP model.

EXTH-58. ONC206, AN IMIPRIDONE FAMILY MEMBER, SUPPRESSES GLIOBLASTOMA CELLS VIA BLOCKING CANCER STEMNESS PATHWAYS

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INTRODUCTION: Imipridones selectively target G protein-coupled receptors (GPCRs) that control critical signaling pathways in various cancer cells. Aberrant overexpression of GPCRs has been implicated in tumorigenesis. ONC201, a first generation imipridone that directly antagonizes dopamine receptor D2 (DRD2), continues to be evaluated in clinical trials for advanced cancers. The immediate downstream mechanism(s) of DRD2 inhibition and resulting anti-cancer activity remains an area of active study. METHODS & RESULTS: ONC206, an analog of ONC201, shares the same imipridone core chemical structure and selective antagonism of DRD2, potently inhibits treatment-resistant glioblastoma caused by intra-tumoral heterogeneity with clinically achievable concentrations. In silico analysis of a glioma patient database revealed that alteration of DRD2 mRNA expression was directly connected to global gene expression change. Imaging DRD2 expression by immunofluorescence demonstrated heterogeneous expression of DRD2 in the glioblastoma cells. After DRD2 inhibition, global metabolite profiling in patient-derived glioblastoma stem cells (GSCs) compared with differentiated glioblastoma cells (DGCs) demonstrated globally differential effects in their cellular signaling pathways. Cell viability assay showed that exposure to ONC206 in a dose dependent manner preferentially eliminated GSCs with 50 to 200 nM of IC50 ranges, whereas the IC50 of DGCs ranged from 200 to 1000 nM. In vitro limiting dilution and sphere formation assay showed that ONC206 prevented tumor sphere formation and tumor growth. ONC206 down-regulated protein expression of cancer-related stem cell markers in the GSCs; silencing DRD2 expression confirmed the dependency of DRD2 expression on cancer stem cell niches in glioblastoma. CONCLUSIONS: ONC206 treatment displays the differential effects on glioblastoma cells, more selectively targeting DRD2 in GSCs (at nanomolar concentrations) compared with DGCs in culture and in xenograft models. This suggests that a therapeutic strategy targeting DRD2-expressing GSCs within glioblastoma may be beneficial for overcoming the therapeutic resistance.

EXTH-59. INTRACRANIAL IMPLANTATION OF TUMORICIDAL STEM CELL-SEEDED SCAFFOLDS AFTER GLIOBLASTOMA RESECTION

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The most common and aggressive primary brain cancer, glioblastoma (GBM), carries a life expectancy of 12–15 months. The short life expectancy is due, in part, to the inability of the current treatment, consisting of surgical resection followed by radiation and temozolomide, to eliminate invasive tumor foci. Treatment targeting invasive tumor foci may be advanced with tumoricidal human mesenchymal stem cells (MSCs). These cells exhibit potent tumor tropism and can be engineered to kill tumor cells in preclinical models. Advancements in preclinical models indicate surgical resection induces premature MSC loss and reduced therapeutic efficacy. Efficacy of MSC treatment can be improved by preloading MSCs

on a biodegradable poly(lactic acid) (PLA) scaffold. MSC delivery on a PLA scaffold restores cell retention, persistence, and tumor killing. To study the effects of MSC-seeded PLA implantation on GBM, an accurate preclinical model is needed. Here we report a preclinical surgical method for performing image-guided tumor resection of GBM in immune-deficient mice followed by MSC-seeded scaffold transplantation. MSCs were engineered with lentiviral constructs to constitutively express and secrete the DR4/5 agonist TNFα-related apoptosis inducing ligand (TRAIL) as well as GFP to allow fluorescent tracking. Similarly, the human GBM tumor cells were engineered to express mCherry and Firefly luciferase, providing dual fluorescent/luminescent tracking capabilities. Using quantitative BLI, we found that maximal resection of visible fluorescent tumor cells failed to fully eradicate the tumor mass. Up to 10% of the pre-operative tumor signal intensity remained post-resection, mirroring observations in human patient testing. MSC-TRAIL implanted on PLA scaffolds were found to significantly suppress tumor recurrence, as animal survival improved 120% over control (13.5 vs 31 days). While used currently for investigating improvements to stem cell mediated delivery of therapeutics, this approach could be modified to research the impact of surgical resection on other therapeutic interventions.

EXTH-61. PARTNERSHIP FOR DEFINING THE IMPACT OF 12 SELECT RARE CNS TUMORS: A REPORT FROM CBTRUS AND THE NCI-CONNECT

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BACKGROUND: Measuring tumor-specific incidence, prevalence, and survival is essential to evaluate the contribution of specific tumor-types to overall cancer burden in the United States population. Central nervous system tumors are rare, with specific tumor types rarer than others. NCI-Connect was developed to understand tumor biology, patient outcomes, and develop standards of care for 12 select rare CNS Tumors. The aim of this study was to determine the incidence, prevalence, and survival of specific rare brain tumors by histology, age, race, and sex in this subset. METHODS: Data derived from CBTRUS was used to examine age-adjusted incidence rate (AAIR) per 100,000 and data derived from SEER was used to examine prevalence and survival statistics from 2000–2014 (all with 95% confidence intervals). The data was then used to compare specific rare brain tumors overall and by sex, age group, race, and ethnicity. RESULTS: The total AAIR was 6.57/100,000 for these specific brain tumor types combined; with the highest AAIR at 0.4/100,000 for oligodendroglioma. Overall, the specified tumors were most incident in males, adults (age 40+), white individuals, and non-Hispanic individuals. Ependymomas were most prevalent at 4.11 cases per 100,000; followed by brain stem and midline gliomas at 1.98 cases per 100,000. Relative survival at 1-, 5-, and 10-years was 59.7%, 33.3%, and 27.7% respectively for all subtypes. Ependymomas had the highest relative survival rate (94.2%, 83.9%, and 78.6%) and gliosarcomas had the lowest relative survival rate (42.5%, 5.6%, 2.9%) at all three time points. CONCLUSION: Overall incidence and prevalence of these brain tumors was very low and varied considerably by each tumor type. Survival also varied by tumor type. The comparison of incidence, prevalence, and survival of rare brain and CNS tumors is critical for measuring the clinical impact of these diseases and for determining future research and clinical care.

EXTH-62. THE DIELECTRIC PROPERTIES OF MALIGNANT GLIOMA TISSUE

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OBJECTIVE: Recently, tumor treating fields (TTFields) have been established as a new treatment for newly diagnosed glioblastoma (GBM). One of the most crucial parameters defining the treatment efficacy of TTFields is the electric field intensity. The electric properties of the normal intracranial compartments are well established, allowing the prediction of the electric field distribution. In contrast, there is no data available about the electric properties of tumor tissue, which leads to a lack of information regarding the electric field intensity within the tumor. In this study we will determine the dieclectric properties of malignant glioma by analyzing resected tissue following a fast acquisition protocol. METHODS: 20 patients with high grade glioma are currently being recruited. Tissue probes are acquired from the infiltration zone, vital tumor area, and perinecrotic compartment. The tissue was analyzed immediately after acquisition to avoid artifacts by temperature change, change of fluid composition as well as post resection ischemia. A cylindrical fragment is dissected from each tissue sample and is placed into a cylindrical cell with a known diameter. The impedance is recorded at frequencies 20Hz-1MHz using a software specifically developed for this study, which controls the LCR meter (Keysight Technologies, Santa Rosa, USA). The measured impedance is then translated into dielectric properties of the sample (conductivity and relative permittivity) based on the parallel plate model, the recorded complex impedance and the geometry of the samples. Each tissue probe will be fixed, H&E stained and histologically analyzed for tumor cell count and specific tissue features (infiltration into normal brain, vital tumor area, necrosis). RESULTS: The study has received a positive vote from the Ethical Board of the University Regensburg Medical Center. The first patients are being recruited, the actual results will be evaluated and presented during the meeting.

EXTH-63. EFFICIENT ADCC-MEDIATED KILLING OF MALIGNANT MENINGIOMA CELLS USING AVELUMAB AND AN ENGINEERED HIGH AVIDITY NATURAL KILLER CELL LINE, haNK

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BACKGROUND: Meningiomas are the most common primary brain tumor. Standard of care includes surgery and radiation therapy. There are no known effective medical therapies for recurrent meningioma, particularly for WHO grades II and III. As such, novel therapeutic approaches are desperately needed. PD-L1 is highly expressed in malignant meningioma, including the cell lines we tested, creating a potential target for ADCC-mediated killing. Therefore, we investigated the ability of avelumab, a PD-L1-specific antibody, to direct NK-mediated ADCC against malignant meningioma cells using healthy donor NK cells and the engineered NK cell line, haNK. METHODS: PD-L1 expression was assayed by flow cytometry and Western blotting in five human malignant meningioma cell lines. Avelumab-targeted ADCC was measured with healthy donor NK and haNK cells. Efficacy of avelumab+haNK was determined *in vivo* against meningioma implanted subcutaneously and orthotopically in a skull-base intracranial model. PD-L1 was deleted from tumor cells using CRISPR knockout to test specificity of the target. RESULTS: Avelumab directed healthy donor NK and haNK cells to mediate ADCC against all five meningioma cell lines *in vitro*. ADCC was enhanced by using NK cells with a high-avidity Fc receptor, haNK cells, or by upregulating PD-L1 in target cells. Avelumab+haNK significantly extended survival in mice bearing orthotopic meningioma and subcutaneous tumors. Conversely, killing (and survival benefit) was abrogated against cells in which PD-L1 was deleted. No toxicity was noted in pre-clinical models. CONCLUSIONS: We demonstrate that avelumab can target meningioma for ADCC by healthy donor NK cells, and killing is significantly enhanced with haNKs. haNK cells have demonstrated safety in humans, and avelumab has shown promising clinical activity in a variety of solid tumors. These data support the design of a clinical trial targeting PD-L1 with avelumab and haNK cells, potentially offering a novel immunotherapeutic approach for patients with malignant meningioma.

EXTH-64. IMIPRIDONES CAUSE METABOLIC REPROGRAMMING AND ELICIT UNIQUE VULNERABILITIES IN PRECLINICAL MODEL SYSTEMS OF GLIOBLASTOMA

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The purpose of this study is to improve the efficacy of imipridones, a novel class of AKT/ERK inhibitors. The lead compound ONC201 has entered clinical testing for glioblastoma (GBM) and recently chemically modified imipridones, ONC206 and ONC212, have been designed. Transcriptome, untargeted liquid chromatography/mass spectrometry and extracellular flux analysis unraveled the mechanism of action of novel imipridone compounds, ONC206 and ONC212. We used orthotopic patient-derived GBM xenografts to assess preclinical treatment efficacy. Imipridones inhibit the prolifer-

ation of patient-derived xenograft and stem-like glioblastoma cell cultures *in vitro* and in multiple xenograft models *in vivo*. ONC212 demonstrated the highest efficacy. High levels of c-myc predict susceptibility to cell death induction by imipridones and increased host survival in orthotopic patient-derived xenografts. At 1h, imipridones inhibit AKT and ERK signaling, accompanied by dephosphorylation of GSK3b. GSK3b phosphorylates c-myc at threonine 58, leading to its proteasomal degradation. Imipridone mediated suppression of c-myc inhibits both glycolysis and oxidative phosphorylation. In turn, energy deprivation leads to a compensatory activation of the serine-one carbon-glycine (SOG) pathway. Interference with the SOG pathway through novel inhibitors of phosphoglycerate dehydrogenase (PHGDH) results in synergistic apoptosis induction *in vitro* and *in vivo*. These observations suggest that c-myc expression predicts therapeutic responses to imipridones and that imipridone mediated inhibition of c-myc leads to suppression of tumor cell energy metabolism, eliciting unique metabolic vulnerabilities that can be exploited for clinical relevant drug combination therapies.

EXTH-65. BET INHIBITION IN COMBINATION WITH TEMOZOLOMIDE TARGETS MYCN-POSITIVE GLIOBLASTOMA CELLS

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Glioblastoma (GBM) is the most common malignant brain tumor in adults with a 15 month median survival after diagnosis. Patients receive surgical resection of the tumor, followed by aggressive radio- and chemotherapy with temozolomide (TMZ). Many human cancers including GBM demonstrate addiction to MYC transcription factor signaling and thus become susceptible to inhibition of MYC downstream genes. JQ1 is an effective inhibitor of BET Bromodomains, a class of epigenetic readers regulating expression of MYC genes and their downstream targets. In a panel of 19 patient-derived GBM cell lines, we showed that BET inhibition alone and in combination with TMZ decreases viability by inducing cell cycle arrest, apoptosis and senescence. We propose a distinct expression signature of GBM cells that correlates with a sensitivity to BET inhibition. Cell lines most sensitive to BET inhibition exhibited ten-fold upregulation of MYCN, as compared to resistant cell lines, while we could not find a correlation between MYC expression and sensitivity to the inhibition. Following BET inhibition, JQ1-sensitive cells downregulated MYCN both on mRNA and protein level, while the inhibition of JQ1-resistant cells had no effect on MYCN expression. In JQ1-sensitive cells, we found enrichment of pathways regulating cell cycle, DNA replication, DNA damage response and repair. As DNA repair leads to an acquired chemoresistance to TMZ, JQ1 treatment in combination with TMZ can further inhibit proliferation of TMZ-resistant cells. Intriguingly, JQ1-sensitive cells expressed high levels of OLIG2 and LGR5, suggesting that BET inhibition targets a subset of proneural GBM progenitors. Taken together, we suggest that BET inhibition can increase the potency of TMZ therapy in particular for GBM patients with a MYCN-positive signature. Ongoing *in vivo* evaluation of JQ1 and TMZ aims to determine the efficacy of the drug combination in inhibiting tumor growth.

EXTH-66. ELUCIDATING THE TRANSLATIONAL POTENTIAL OF LA_2O_3 NANOPARTICLES AS A NOVEL THERAPY IN GLIOBLASTOMA

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BACKGROUND: Given the current outlook for glioblastoma (GBM) is dismal, novel therapies warrant consideration. Lanthanum oxide (La_2O_3) nanoparticles (NPs) represent a nanotechnology with greater ability to penetrate the blood brain barrier (BBB), induce a natural anti-cancer effect, and enhance current treatment modalities. The aim of this study was to first validate and describe the therapeutic effect of La_2O_3 NPs in GBM *in vitro*, and then explore its translational potential *in vivo*. METHODS: La_2O_3 NPs were first tested for cytotoxicity, synergy with temozolomide (TMZ) and radiosensitization *in vitro* in 4 GBM patient derived cell lines (PDCLs). Mechanisms were then explored using electron microscopy, flow cytometry, and western blotting. Finally, *in vivo* studies were conducted in balb/c mice with xenograft GBM PDCL models to establish translational potential. RESULTS: There was significant cytotoxic effect, synergy with TMZ and radiosensitization observed with La_2O_3 NPs in all PDCLs tested. Analysis indicated that the NPs enter the cell via clathrin-mediated endocytosis and cause intracellular calcium and radical oxygen species imbalance. This triggers mitochondrial apoptotic mechanisms and augments TMZ and radiation effect. *In vivo*, detectable levels of La_2O_3 NPs were observed in the xenograft model tumors after intravenous administration of maximal tolerable dose without any serious adverse events. CONCLUSION: La_2O_3 NPs represent a novel therapy which can ameliorate the dismal prognosis of GBM. *In vitro* and *in vivo* modelling thus far indicates that it does have translational potential in penetrating the BBB to reach glioblastoma and cause anti-cancer effect as a single agent, and in combination with current management strategies. Considerations for *in vivo* survival studies were thus justified, and are currently underway.

EXTH-67. HIGH-DOSE METFORMIN PLUS TEMOZOLOMIDE SHOWS INCREASED ANTI-TUMOR EFFECTS IN GLIOBLASTOMA IN VITRO AND IN VIVO COMPARED WITH MONOTHERAPY

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Glioblastoma (GBM) is one of the most aggressive cancers and the most common primary brain tumor. Although concomitant chemoradiotherapy using temozolomide (TMZ) has been established as the standard therapy, the median survival is approximately 14 months. Metformin, an anti-diabetic agent, has been reported to have anti-tumor effects in various cancers. The purpose of the study is to investigate the efficacy of combined treatment with TMZ and metformin for the treatment of GBM *in vitro and in vivo*. We investigated the efficacy of combined treatment with TMZ and metformin compared with monotherapy using cell viability and apoptosis assays. A GBM orthotopic mice model was established and treated with metformin, TMZ, and the combination. Survival data were recorded, and tumor specimens were analyzed using western blotting and immunofluorescence. The combination of TMZ and metformin showed higher cytotoxicity than single agents in U87, U251, and A172 cell lines. A combination of high-dose metformin and TMZ showed the highest apoptotic activity. The combination of TMZ and metformin enhanced AMPK phosphorylation and inhibited mTOR phosphorylation and p53 expression. The median survival was 43.6, 55.2, 53.2, 65.2, and 71.3 days in control, metformin (2 mg/25 g/day), TMZ (15 mg/kg/day), combination treatment of low-dose metformin (2 mg/25 g/day) and TMZ (15 mg/kg/day), and combination treatment of high-dose metformin (10 mg/25 g/day) and TMZ (15 mg/kg/day), respectively (p=0.001). Expression of fatty acid synthase (FASN) was significantly decreased in tumor specimens treated with metformin (10 mg/25 g/day) and TMZ (15 mg/kg/day). The combination of metformin and TMZ was superior to monotherapy using metformin or TMZ in terms of cell viability *in vitro* and survival *in vivo*. The combination of high-dose metformin and TMZ inhibited FASN expression in an orthotopic model. Inhibition of FASN might be a potential therapeutic target of GBM.

EXTH-68. ZIKA VIRUS ONCOTROPISM TOWARDS GLIOBLASTOMA PROGENITOR CELLS IS MEDIATED BY THE TYROSINE KINASE RECEPTOR Axl

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An oncolytic virus must display tropism for the target cancer. This oncotropism is mediated by cell surface receptors that are differentially expressed by the cancer cell. Recent research into the mechanism of Zika virus (ZV) induced microcephaly demonstrated that ZV has neurotropism for fetal neural progenitor cells, and this is mediated by Axl. Axl is a member of the TAM (Tyro3, Axl, Mer) family of tyrosine kinase receptors. Additional research showed that Axl is overexpressed in 60% of glioblastoma (GBM). We therefore hypothesized that ZV could target GBM progenitor cells through the Axl receptor. Using Western blot, we discovered that there is a range of Axl expression in commercially available cell lines (5 GBM, 2 breast cancer) and in 11 patient derived GBM progenitor cell lines. Using flow cytometry and immunofluorescence microscopy, we confirmed that ZV cell entry correlates with Axl expression. ZV did not enter nor productively infect Axl negative cell lines. While at 24 hours post exposure, the number of intracellular viral particles increased as Axl expression increased. We next demonstrated that ZV entry was abrogated by the Axl inhibitor R428 in a dose dependent manner, and, anti-Axl antibody treatment also prevented ZV entry. These findings were again confirmed with both flow cytometry and immunofluorescence microscopy. We therefore conclude that ZV is oncotropic towards GBM progenitor cells and this is mediated by the Axl receptor. Thus, ZV meets the first requirement for a GBM oncolytic virus: receptor mediated oncotropism.

EXTH-69. MAGNETIC HYPERTHERMIA THERAPY OF EXPERIMENTAL GLIOBLASTOMA IN COMBINATION WITH CHEMORADIATION

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INTRODUCTION: Fractionated radiation therapy (RT) combined with chemotherapy (temozolomide; TMZ), known as chemoradiation (CRT), has provided the greatest benefit to glioblastoma (GBM) patients. Magnetic hyperthermia therapy (MHT) consists of intratumoral heat generation after deposition of magnetic iron-oxide nanoparticles (MIONPs) that are subjected to an external alternating magnetic field (AMF). MHT may potentiate the cytotoxic effects of CRT on tumor cells. We have developed MIONPs that demonstrate heating efficacy for MHT. METHODS: Intracranial heating efficacy and toxicity studies were performed in healthy mice using MIONPs solutions with a clinically relevant AMF treatment (450kHz, 200G) after convection-enhanced delivery (CED). Bioluminescence imaging (BLI) was used to assess the in vivo efficacy of intracranial MHT in combination with fractionated RT in a mouse syngeneic GBM model. A pilot survival study of athymic nude mice with invasive, therapy-resistant orthotopic human GBM intracranial xenografts was completed after combination treatment with MHT and CRT. Quantification of TMZ levels in intracranial xenografts and surrounding brain tissue after MHT was performed using liquid chromatography–mass spectrometry (LC-MS). RESULTS: A concentration-dependent temperature increase by the MIONPs after CED and an AMF was observed rapidly in the brain. No temperature elevation was observed in either the contralateral brain hemisphere or the rectum, supporting the localized intracranial heating effect. No severe acute and long-term side effects were observed after MIONP CED. A marked decrease in tumor size was found with MHT and RT by BLI. Significantly prolonged animal survival occurred with MHT and CRT in comparison to monotherapy. Increased TMZ levels were measured in xenografts and surrounding brain tissue after MHT and TMZ treatment. CONCLUSIONS: We have confirmed the safety and feasibility of intracranial MHT in a rodent glioma model. Furthermore, we have demonstrated the chemoradiosensitivity enhancement of therapy-resistant human GBM xenografts after MHT.

EXTH-70. EFFICIENT DELIVERY OF SIRNAS TO GLIOMA VIA FUNCTIONALIZED EXTRACELLULAR VESICLES PRIMED BY RADIATION

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Efficient delivery is a major obstacle in oligonucleotide therapeutics for the treatment of gliomas. Endogenous small vesicles known as extracellular vesicles (EVs) hold potential to act as oligonucleotide delivery vehicles given their unique properties, such as low immunogenicity, innate stability, and ability to cross the blood-brain barrier (BBB). However, their insufficient targeting ability upon intravenous administration limits the clinical advancement. In this study, neural stem cell line ReNcell was cultured to release EVs. The cyclo(Arg-Gly-Asp-D-Tyr-Lys) peptide [c(RGDyK)], which exhibits high affinity to integrin αvβ3 on tumor vascular endothelial cells, was conjugated on the isolated EVs via bio-orthogonal click chemistry. In the syngeneic graft glioma mice model, the c(RGDyK)-conjugated EVs (cRGD-EV) targeted glioma after intravenous administration. Then, priming with radiation enhanced cRGD-EV accumulation in the tumor and decreased their entrapment in the liver and spleen significantly. Furthermore, small interfering RNAs (siRNAs) for immune checkpoint molecules programmed death-ligand 1 (anti-PD-L1) and CD47 (anti-CD47) were conjugated with cholesterol, and loaded into EV membrane by hydrophobic interaction simultaneously. After intravenous injection, the knockdown of target proteins in the tumor was found. Also, the number of infiltrated CD4+ and CD8+ T cells and M1/M2 rate of macrophages were increased, showing the up-regulated immune response in tumor. The injection of siRNAs-loaded cRGD-EV induced the regression of tumor growth in immuno-competent mice and significantly prolonged survival. In addition, no obvious liver and lung toxicity or tissue damage was observed in the treated mice. These results suggest a targeting delivery strategy for glioma based on EVs, and have implications for cancer immunotherapy.

EXTH-71. RADIATION-INDUCED TARGETED NANOPARTICLE-BASED GENE DELIVERY FOR BRAIN TUMORS

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Targeted therapy and programmed cell death ligand-1 blockade hold a great promise for the treatment of different aggressive tumor types, however little effect have been observed against gliomas probably due to inability of large antibodies in penetrating the brain. An effective glioma therapy requires a delivery system that reaches tumors in the central nervous system, with limited systemic effect. In this study, we developed an iRGD-based solid lipid nanoparticle (SLN) to deliver siRNAs against both EGFR and PD-L1 for combined targeted and immunotherapy against glioblastoma, the most aggressive type of brain tumors. Building on recent studies showing that radiation therapy alters tumors for enhanced nanotherapeutic delivery in tumor-associated macrophages-dependent fashion, we showed that low dose radiation primes an increase in targeted SLN uptake into the brain tumor region leading to enhanced downregulation of PD-L1 and EGFR. Bioluminescence imaging revealed that radiation therapy followed by systemic administration of targeted SLN lead to a significant decrease in glioblastoma growth, and prolonged mouse survival. This study combines radiation therapy to prime the tumor for nanoparticle uptake along with the targeting effect of iRGD conjugated nanoparticles to yield a straightforward but effective approach for combined targeted EGFR inhibition and immunotherapy against glioblastomas, which can be extended to other aggressive tumor types.

EXTH-72. MP-Pt(IV): A MAOB SENSITIVE MITOCHONDRIAL SMART BOMB FOR TREATING GLIOMA

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We have previously demonstrated that monoamine oxidase B (MAOB), highly elevated in glioma, may be used to catalytically convert uncharged methyl-tetrahydropyridine (MP-) groups into the mitochondrial targeted, cationic, methyl-pyridinium (P^+-) form. Our first generation mitochondrial 'smart-bomb', MP-MUS, used a nitrogen mustard 'warhead', and was demonstrated to attach glioma mitochondrial DNA *in vitro* and *in vivo*. The development of our lead compound into a clinically used therapeutic was hampered by the reactivity of the nitrogen mustard 'warhead' and thus the potential of off-target toxicity. We now report our second generation lead compound, MP-Pt(IV), in which we replaced the reactive nitrogen mustard with an unreactive platinum (IV) group. This new 'warhead' was designed to undergo ascorbate-linked reductive activation so as to give rise to *cis*-platin, DNA-crosslinking chemotherapeutic, that will be able to attack gliomal mitochondrial DNA. MP-Pt(IV) is a very good substrate for MAOB, but not MAOA, and is converted by gliomal MAOB into the cationic, lipophilic mitochondrial targeting P^+-Pt(IV). The principle biological reductant capable of converting Pt(IV) into *cis*-platin is ascorbate. We show that gliomas have high levels of mitochondrial ascorbate, and these elevated levels of ascorbate reflect the elevation of glucose/dehydroxyascorbate transporters, GLUT1, GLUT 3, and GLUT4. Here we show that MP-Pt(IV) is highly effective chemotherapeutic, in *in vitro* as well as *in vivo* mouse intracranial mouse models of glioblastoma. *In vitro* studies show that we can potentiate the toxicity of MP-Pt(IV) by increasing mitochondrial ascorbate levels, by incubating cells with dehydroxyascorbate. In an *in vivo model*, we see that MP-Pt(IV) potentiates the classical chemotherapeutic agent temozolomide and also temozolomide based chemoradiation. Of note is the ability of MP-Pt(IV), like MP-MUS, to cause an elevation in mitochondrial, MAOB levels in treated cells. Treatment of glioma with MP-Pt(IV) creates the opposite of drug resistance, with treating cells becoming increasingly sensitive to MP-Pt(IV).

EXTH-73. TARGETING THE SWEET-TOOTH OF GLIOMA

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It has long been recognized that sugar transporters are often upregulated in cancers and the upregulation of the Glut3 glucose/galactose transporter in glioma is well described. We have identified that Glut14, the testicular-specific glucose/galactose transporter is also upregulated in GBM, and that levels of Glut3/Glut14 predict patient outcome. We used a Glut3/Glut14 substrate and glucose/galactose analogue, 4-deoxy 4-fluoro Galactose (4D4FG), to inhibit glycan synthesis in glioma, *in vitro* and *in vivo*. As 4D4FG is a potent inhibitor of both UDP-glucose 4-epimerase (GALE) and UDP-glucose 6-dehydrogenase (UGDH), we were able to observe aberrant glycan synthesis in glioma exposed to 4D4FG. Perturbation of glycan synthesis correlated with gliomal cell death *in vitro*, and is accompanied by loss of nuclear localization of glycosylated proteins. In a flank mouse model of primary GBM we compared the effects of three cycles of *i.p.* 4D4FG with vehicle. We found that 4D4FG initially caused a small shrinkage of tumors, and then slowed tumor growth by 35%, with respect to the controls. Using the upregulation of gliomal sugar transporters to differentially target cancer glycan metabolism is clearly deleterious to glioma. Anti-metabolites like 4D4FG will never be first-line chemotherapeutics in cancer treatment. However, such anti-metabolites may be very usefully used to pre-stress glioma, prior to chemoradiation, to differentially sensitize the tumor to conventional treatment.

EXTH-74. MOLECULAR MECHANISMS OF ANTI-TUMOR ACTION OF TTFIELDS DETERMINED BY MEASUREMENTS AND MODELING OF ELECTRO-CONDUCTIVE PROPERTIES OF MICROTUBULES

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Biological effects of AC electric fields at frequencies between 100–300 kHz discovered a decade ago are being applied to cancer cells as a therapeutic modality in the treatment of glioblastoma multiforme (GBM). They are called Tumor Treating Fields (TTFields) as they disrupt cell division. Based on our electro-conductive measurements and modeling, we provide an assessment of possible molecular-level mechanisms. Computer simulations and experimental measurements carried out for microtubules and actin filaments are presented. Charge and dipole values for monomers and dimers as well as polymerized forms of these proteins are summarized. Continuum approximations for cable equations describing actin filaments and microtubules compare favorably to measurements in buffer solutions showing soliton waves and transistor-like amplification of ionic signals, respectively. AC Conductivity and capacitance of tubulin and microtubules have been measured and modeled in the range of frequencies between 100 kHz and 1 MHz. A dramatic change in conductivity occurs when tubulin forms microtubules. In living cells, this signals a conductive phase transition coinciding with mitosis in dividing cells. This process is allowed by TTField penetration into the cleavage furrow in dividing cells and provides the most significant mechanistic explanation of the observed effects. We provide estimates of the forces, energies and power involved in the action of TTFields on microtubules and kinesin motors. These calculations are compared and contrasted with typical values experienced at a cell level and provide strong arguments for real physical effects of TTFields in dividing cells. We also show results of DLS and TEM measurements on microtubules and tubulin oligomers in solution, which allow us to quantify these processes under controlled conditions. In conclusion, the most likely candidates to provide a quantitative explanation of these effects are ionic condensation waves around microtubules as well as dielectrophoretic effects on the dipole moments of microtubules.

EXTH-75. THE AURORA KINASE A INHIBITOR ALISERTIB IS SYNERGISTIC WITH IRINOTECAN AND CARBOPLATIN AGAINST GLIOBLASTOMA CELLS

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INTRODUCTION: Alisertib is a selective AURKA inhibitor currently in clinical trial for recurrent glioblastoma. We have previously shown that alisertib potently inhibits growth of glioblastoma cells *in vitro* and *in vivo*. As effective chemotherapeutic approaches for refractory glioblastoma may require a combination of agents, we tested the efficacy of alisertib to potentiate the effects of carboplatin and irinotecan. METHODS: The ability of alisertib to potentiate the growth inhibitory effects of these agents was assessed by using colony formation assays with cultured glioblastoma cells. RESULTS: Alisertib potentiated the anti-proliferative action of both irinotecan and carboplatin in these assays. This effect was often synergistic, including against glioblastoma tumor stem-like cells (neurospheres), as demonstrated by Chou-Talalay and Bliss analyses. We then compared *O*^{6}*-methylguanine DNA methyltransferase* (MGMT) expression levels of each cell line by western blotting, and found that high MGMT expression correlated with more pronounced potentiation of carboplatin's growth inhibitory effects by alisertib, while low MGMT expression correlated with stronger potentiation of irinotecan by alisertib. This suggests that MGMT expression levels may be predictive of patient response to these drug combinations, however additional studies are required to confirm this possibility. CONCLUSIONS: Since clinical experience with alisertib, carboplatin and irinotecan as single agents already exists, these findings may provide rationale for the design of clinical trials for their use in combination treatment regimens.

EXTH-76. ^{1}H AND HYPERPOLARIZED ^{13}C MRS BIOMARKERS OF IDH1 MUTANT GLIOMA RESPONSE TO TEMOZOLOMIDE THERAPY

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The alkylating agent temozolomide (TMZ), previously reserved for treatment of glioblastoma, is now being considered for the treatment of low-grade glioma that are driven by mutations in the isocitrate dehydrogenase 1 (IDH1) gene. Though the treatment of IDH1 mutant patients with TMZ improves survival, there is a need for metabolic imaging to help in assessing early response to therapy. The goal of this study was, therefore, to determine the value of magnetic resonance spectroscopy (MRS)-based biomarkers for detection of response to treatment. To address this, we examined the global metabolic alterations that occurred following TMZ treatment in a genetically engineered IDH1 mutant immortalized normal human astrocyte cell line (NHAIDHmut) using ^{1}H and ^{13}C MRS combined with chemometrics. Cells were treated either with the IC50 of TMZ (100 μM; N=5), or with DMSO (0.2%; N=5) for 72 hours. Then, metabolites were extracted from cells and ^{1}H spectra acquired. Data were analyzed using SIMCA and the most significant metabolites contributing to class separation were identified using multivariate and univariate analyses. Alternatively, live cells were exposed to hyperpolarized 2-^{13}C-pyruvate and dynamic sets of ^{13}C-MRS spectra recorded to monitor the production of 5-^{13}C-glutamate over time. ^{1}H MRS showed that glutamine, glutamate, pyruvate, succinate, glucose, phosphocholine, isoleucine, valine, lysine, phenylalanine, NAD+/NADP+ and ATP/ADP/AMP were significantly higher in TMZ-treated cells as compared to controls. Accordingly, the tricarboxylic acid (TCA) cycle was identified as a significantly altered pathway following TMZ treatment. Consistent with this finding, dynamically probing the metabolism of hyperpolarized 2-^{13}C-pyruvate revealed that build-up of 5-^{13}C-glutamate, which is associated with flux to the TCA cycle, was significantly higher in TMZ-treated cells as compared to controls. Further studies are warranted for validation of our findings in other mutant IDH1 models. Nonetheless, our findings identify potential MRS-detectable early biomarkers of response to TMZ therapy in mutant IDH1 glioma.

EXTH-77. PARP INHIBITION STRIKINGLY ENHANCES CHEMOTHERAPEUTIC EFFECT IN CHORDOMA

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BACKGROUND: Therapy for chordoma, a cancer of the axial skeleton, consists of surgery often followed by high-dose irradiation. There are no known effective medical therapies. Because at recurrence, surgery and irradiation options are often limited, there is a critical unmet need to develop effective medical therapeutic strategies. METHOD: In the present study, we investigated three patient-derived chordoma cells for molecular mechanisms of underlying therapeutic resistance. *In vitro* high throughput chemical screening assay, as well as *in vivo* xenograft model, was used to identify novel chemo-sensitizers for chordoma cells. RESULTS: We found that chordoma cell lines recapitulate disease phenotype, highlighted with robust therapy resistance and lack of DNA damage accumulation. Mechanistically, PARP DNA repair pathway plays central roles in the chemotherapeutic resistance. High throughput chemical screening confirmed that PARP inhibitors remarkably enhanced genotoxicity in chordoma cells. Combining an FDA-approved inhibitor olaparib not only potentiates DNA damage accumulation, cell cycle arrest, apoptotic changes *in vitro*, but also strikingly suppressed chordoma xenograft expansion *in vivo*. CONCLUSION: We conclude that combining PARP inhibitor with genotoxic therapy could be an effective therapeutic approach for the clinical management of chordoma.

EXTH-78. PARP INHIBITION IMPROVES RADIOTHERAPY EFFECTIVENESS IN MENINGIOMA CELL CULTURE.

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INTRODUCTION: Medical therapies and radiological adjuvants are currently limited for aggressive meningiomas. Inhibitors of poly (ADP-ribose) polymerase (PARP), such as ABT-888, plays a role in cancer by preventing DNA repair. PARP inhibitors can improve sensitization to radiation and alkylating treatments in gliomas and other tumors but have not yet been studied in meningiomas. We hypothesize that ABT-888 and radiation therapy combine to show synergistic anti-tumor effects in meningioma cell lines. Furthermore, we hypothesize that this effect is mediated by hypoxia inducible factor 1A (HIF1A). METHODS: A primary meningioma cell line developed by our lab (GAR-Neg) along with a cell line with a shRNA-HIF1A knockdown (GAR1589) were used. After treatment with ABT-888, TMZ and radiotherapy, cells were evaluated using a cell viability assay (Cell Titer-Glo) and real-time cell microscopy (e.g. Incucyte). Experiments were

performed in triplicate and statistically analyzed. RESULTS: TMZ (3.125 uM, 1.563 uM, 0.75 uM) and ABT-888 (3.125 uM, 1.563 uM, 0.75 uM, and 0.1 uM) significantly reduced viability and proliferation of GAR-Neg cells ($p<0.05$, One-way ANOVA). While TMZ inhibited GAR-Neg cells in a dose-dependent manner (3.125 uM, 1.563 uM, 0.75 uM), PARP (3.125 uM, 1.563 uM, 0.75 uM, and 0.1 uM) resulted in varying effects on viability. Combination therapy with TMZ and ABT-888 showed synergistic effects in combination compared to either dose individually ($p<0.05$). Combination of ABT-888 and radiotherapy showed additive effects when evaluated with real-time cell microscopy. GAR1589 cell showed a modest increase in sensitivity to certain combined drug doses. CONCLUSION: TMZ and ABT-888 combinations showed synergistic anti-tumor effects while radiotherapy and ABT-888 combinations showed additive anti-tumor effects in meningioma cell lines. These results suggest improved methods for combination, targeted treatment of patients with meningioma with lower overall doses of toxic therapies. In addition, HIF1A may play a role in promoting resistance to combined treatments.

EXTH-79. BEVACIZUMAB, IRINOTECAN, TEMOZOLOMIDE, TYROSINE KINASE INHIBITION, AND MEK INHIBITION ARE EFFECTIVE AGAINST PLEOMORPHIC XANTHOASTROCYTOMA REGARDLESS OF V600E STATUS

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BACKGROUND: Pleomorphic xanthoastrocytoma (PXA) is a rare Grade II and III glioma. Surgical resection is the mainstay of treatment; however, adjuvant therapy is sometimes necessary. Given the rarity of PXA, chemotherapeutic efficacy data is limited. The importance of the BRAF V600E mutation in the context of MAP kinase pathway inhibition is unknown. The purpose of this study was to perform an *in vivo* screen of a variety to agents to determine efficacy against both V600E mutant and non-mutant PXA. METHODS: The efficacy of bevacizumab, temozolomide, lomustine (CCNU), irinotecan (CPT 11), a tyrosine kinase inhibitor (sorafenib), a selective MEK1/2 inhibitor (cobimetinib), and a BRAF inhibitor (vemurafenib) were assessed in two subcutaneous xenografts: D645 PXA (V600E-mutant) and D2363 PXA (V600E-non-mutant) (n=5–10 mice). Select agents were also assessed in an intracranial model of D2363 PXA (n=6–9). Subcutaneous tumor growth and survival were the endpoints. RESULTS: Temozolomide, bevacizumab, CPT 11, and sorafenib significantly inhibited subcutaneous tumor growth in both V600E-mutant and V600E-non-mutant models ($P<0.05$). MEK inhibition (cobimetinib) but not BRAF inhibition (vemurafenib) also inhibited tumor growth regardless of V600E mutation ($P<0.05$). Temozolomide, CPT 11, and bevacizumab also prolonged survival in V600E-negative intracranial model (median overall survival (OS) 68.5, 62.5, and 42.5 days, respectively) in contrast to controls (31.5 days, $P<0.001$). CONCLUSION: These findings suggest that when adjuvant treatment is clinically indicated for PXA, temozolomide, CPT 11, sorafenib, or bevacizumab may be considered. Additionally, a trial of a MEK inhibitor could be considered for PXA regardless of V600E mutation status.

EXTH-80. IMPAIRED PARP1 DNA REPAIR DEFINES CHEMO-SENSITIVITY IN IDH1-MUTATED CELL

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BACKGROUND: Mutations in isocitrate dehydrogenase (*IDH1/2)* are the most prevalent genetic deficiency in lower grade gliomas. *IDH*-mutated in glioma exhibits better clinical outcomes with longer patient survival, as well as greater sensitivity to chemotherapy. In the present study, we explored the molecular mechanisms that determine the chemosensitivity in *IDH1*-mutated cells, and seek a potential therapeutic strategy by targeting PARP/BER DNA repair pathway. METHODS: We investigated transcriptomic profiles from 530 WHO grade II/III glioma based on their *IDH1/2* mutation status. We established *IDH1*-mutated cells and investigated the alterations in nicotinamide adenine dinucleotide (NAD^+), Poly (ADP-ribose) polymerase (PARP)-associated DNA repair and DNA damage in these cells in response to temozolomide (TMZ). Moreover, we evaluated the PARP inhibitor olaparib and its synergistic effect on TMZ-associated cytotoxicity. RESULTS: Our results showed that the *IDH1*-mutated cells are more vulnerable to genotoxic agent, which recapitulate the disease phenotype in *IDH1*-mutated glioma. TMZ treatment resulted in an over 20-fold increase of cell death in *IDH1*-mutated cells compared with wild type counterpart. *IDH1*-mutated cells exhibited an over 1.3-fold DNA damage and a 1.42-fold increase in cellular apoptosis with TMZ treatment. Mechanistically, *IDH1*-mutated cells exhibited compromised NAD^+ metabolism, as well as concomitant PARP/BER DNA repair pathway. *IDH1*-mutated cells produced 83.8% less poly (ADP-ribose) polymer (pADPR), an important substrate for PARP-associated DNA repair, during TMZ treatment. This suggests that their incompetence to maintain genomic integrity was due to decreased availability of NAD^+. Targeting the PARP-associated DNA repair pathway using olaparib, an FDA-approved PARP inhibitor, remarkably potentiated TMZ-induced cytotoxic effects by 2.16-fold in *IDH1*-mutated cells. CONCLUSION: Our findings demonstrate that metabolic defects in *IDH1*-mutated cells affect PARP-associated DNA repair pathway via NAD^+ depletion, and therefore prompt the sensitivity to chemotherapies. Targeting PARP-associated DNA repair pathway suggests a novel therapeutic avenue for *IDH1*-mutated gliomas.

EXTH-81. TARGETING Nrf2 ANTIOXIDATIVE PATHWAY AS A NOVEL THERAPEUTIC STRATEGY FOR IDH1-MUTATED CANCERS

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BACKGROUND: Mutations in isocitrate dehydrogenase (*IDH1/2)* are the most prevalent genetic abnormalities in lower grade glioma. Neomorphic enzyme activity of mutant IDH1/2 leads to the production of oncometabolite 2-hydroxyglutarate, accompanied with disruption of redox balance through aberrant $NADP^+$/NADPH metabolism. The remarkable accumulation of reactive oxygen species (ROS) suggests distinctive metabolic stress and vulnerability in cancer cells, implying the potential for selective therapeutic approaches for *IDH1*-mutated malignancies. METHODS: We investigated the ROS homeostasis in *IDH1* mutation transduced cells as well as patient derived *IDH1*-mutated brain tumor initiating cells (BTIC). The importance of antioxidant genes was confirmed through COX regression analysis from a large cohort of *IDH1*-mutated lower grade glioma. Further, we investigated the involvement of Nrf2, the master transcriptional factor that regulates antioxidant enzymes in *IDH1*-mutated cells. Finally, we evaluated the therapeutic value of Nrf2 inhibitor in *IDH1*-mutated cancer *in vitro* and *in vivo*. RESULTS: We found that pathogenic *IDH1* mutation leads to substantial reprogramming in ROS homeostasis, highlighted that this prompted ROS generation and compensatory up-regulation of ROS scavenging genes. The expression of key ROS scavenging genes not only establishes resistance for cancer cells, but also predicts poor disease outcome in *IDH1*-mutated lower grade glioma. Further, *IDH1*-mutated glioma develop dependency on Nrf2-governed antioxidant pathway, which plays a key role in modulating glutamate/glutathione metabolism, ROS detoxification and cancer cell survival. Pharmacologic targeting of Nrf2 not only led to ROS-derived cytotoxicity in *IDH1*-mutated cells, but also selectively suppressed *IDH1*-mutated xenograft growth *in vivo*. CONCLUSION: Our findings showed that Nrf2 antioxidant pathway plays a central role in the biology of *IDH1*-mutated glioma. Targeting Nrf2 antioxidant pathway showed promising tumor suppressing effect, suggesting novel therapeutic approaches for *IDH1*-mutated malignancies.

GENETICS AND EPIGENETICS

GENE-01. THE GENOMIC LANDSCAPE OF TRIPLE-NEGATIVE GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and deadly primary malignant brain tumor in adults. Mutations in the *TERT* promoter (*TERTp)* and isocitrate dehydrogenase 1 or 2 (*IDH1/2*) can classify ~80% of GBMs into molecular subgroups with distinct clinical courses. These molecular subgroups utilize distinct genetic mechanisms of telomere maintenance, either *TERTp* mutation leading to telomerase activation or *ATRX*-mutation leading to an alternative lengthening of telomeres phenotype (ALT). However, approximately 20% of GBMs lack alterations in *TERTp* and *IDH1/2*. These tumors, designated $TERTp^{WT}$-IDH^{WT} or triple-negative glioblastomas (as they also lack 1p19q co-deletion) do not have well-established genetic biomarkers or defined mechanisms of telomere maintenance. Here we performed whole-exome, whole-genome, and RNA-sequencing on a cohort of triple-negative GBMs to define their genetic landscape. We discovered recurrent inactivating mutations in SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like 1 (*SMARCAL1*) in 21% (8/39) of triple-negative GBMs. Using telomere maintenance characterization assays, we show that *SMARCAL1*-mutant cases exhibited the ALT phenotype. Using CRISPR/Cas9 gene editing in GBM cell lines, we found that inducing loss of SMARCAL1 generates features of ALT. Rescue

of expression of SMARCAL1 in *SMARCAL1*-null cell lines markedly suppressed ALT features and was dependent on the enzyme helicase activity. Furthermore, using break-apart FISH and whole genome sequencing, we identified recurrent rearrangements upstream of *TERT* in ~50% of triple-negative GBMs. These *TERT*-rearranged tumors exhibited elevated levels of *TERT* mRNA expression. This represents a novel mechanism of telomerase activation in GBMs lacking the well-known *TERT* promoter hotspot mutations. Finally, we identify recurrent BRAF V600E mutations in younger patients with GBM. Collectively, our findings define novel molecular subgroups of glioblastoma, including a telomerase-positive subgroup driven by *TERT*-structural rearrangements (IDH^{WT}-$TERT^{SV}$, ~50%), and an ALT-positive subgroup (IDH^{WT}-ALT, ~40%) with mutations in *ATRX* or *SMARCAL1*. We also establish *SMARCAL1* inactivating mutations as a novel genetic mechanism of ALT in cancer.

GENE-03. WILD-TYPE Tp53 UNDERGOES A CELL TYPE-SPECIFIC INACTIVATION BY SIRT1 IN DE NOVO GLIOBLASTOMA

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In contrast to most solid tumors, the majority (72%) of *de novo* glioblastoma (GBM) retain a wild-type Tp53 gene while undergoing loss of CDKN2A (INK4a/Arf locus). Remarkably, transcriptional and proteomic data suggest that Tp53 is abundantly expressed in *de novo* GBM; however, it appears not to confer any survival advantage. The possibility that wild-type Tp53-expressing cells do not contribute to tumor growth seems unlikely since Tp53 is even more expressed in a paired analysis of initial and recurrent GBM. Taken together, these observations raise the possibility that the canonical Tp53 signaling may be functionally inactive in *de novo* GBM. Using primary cultures of conditional murine CDKN2A/B neural stem cells (NSC) and astrocytes we found that wild-type Tp53 is abundantly expressed following sequential loss of p16, p15 and p19 in astrocytes but not NSC. Astrocytes that lack both INK4a/b/Arf andTp53 show no growth advantage over INK4a/b/$Arf^{-/-}$ alone. Stable overexpression of Tp53 impairs proliferation of INK4a/b/$Arf^{-/-}$ NSC but not astrocytes, raising the intriguing possibility that inactivation of Tp53 signaling is cell type-specific. Furthermore, using Nutlin our data suggests that the Tp53-MDM2 axis is functional only in NSC but not astrocytes. Analysis of post-translational modifications (PTM) of Tp53 protein in INK4a/b/$Arf^{-/-}$ astrocytes and NSC reveals that it is entirely unacetylated in the former but not in the latter. Similarly, Tp53 expressed in primary GBM cells and patient-derived xenografts is also unacetylated. Consistent with this PTM, we found that SIRT1 is highly expressed in INK4a/b/$Arf^{-/-}$ astrocytes and not NSC and that inhibition of SIRT1 restores Tp53 acetylation, reduces cell proliferation and tumor growth. Mechanistic studies suggest that SIRT1 inhibition restores expression of Tp53 target genes (p21, PUMA, BAX). Taken together, our results identify a previously unrecognized cell type-specific PTM that inactivates Tp53 and provide a rationale for SIRT1 inhibition to restore Tp53 function.

GENE-04. CHARACTERISTICS OF PATIENTS WITH A PRIMARY BRAIN TUMOR UNDERGOING HEREDITARY CANCER MULTI-GENE PANEL TESTING

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BACKGROUND: Currently, no germline genetic testing guidelines exist for patients with a primary brain tumor (PBT). Patient-specific characteristics of PBT patients in relation to genetic etiology are understudied; therefore we aimed to describe these variables based on hereditary cancer multi-gene panel test (MGPT) results among patients with a PBT. METHODS: A total of 654 consecutive subjects were referred for MGPT at a diagnostic laboratory between March 2012-June 2016. Clinical data were ascertained from test requisition forms. Statistical analyses were completed using STATA (v.13, College Station, TX). RESULTS: In our cohort, 67% (339/506) were diagnosed under 50 years of age. MGPT identified 104/654 (16%) patients with germline mutations. Of these, 35 (34%) had an isolated PBT with no additional primary cancers. Half of identified genes predisposed a risk to PBTs, while the other half did not. Test result positive astrocytomas were diagnosed at significantly younger ages than patients with negative and variant of uncertain significance results (p=0.021). No association between tumor grade and genetic testing outcome was observed. Of 165 patients with available family history, 95% (n=157) reported a family history of some cancer and 18% (n=30) reported family history of brain tumors. CONCLUSIONS: While no germline genetic testing criteria currently exist for PBTs, our data suggest younger age of diagnosis is being utilized as an indicator for testing. Particular PBT pathologies have been associated with specific hereditary cancer syndromes; therefore, pathology can be helpful in narrowing down the differential diagnosis. In addition, more research is necessary to better understand the relationship between tumor grade and testing outcomes. Family history evaluation of both PBTs and systemic cancer is a beneficial risk assessment tool, particularly until testing criteria are developed. Further research is necessary for the development of solidified genetic testing criteria in the PBT population and more robust identification of at-risk individuals.

GENE-05. UPREGULATION OF ODZ1-MEDIATED INVASION IN THE HYPOXIC TUMOR MICROENVIRONMENT IN GLIOBLASTOMA

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Differentiation of GBM stem-like cells activates an ODZ1-associated pathway, involving activation of the RhoA-ROCK cascade, leading to cytoskeletal remodelling and invasion into the surrounding environment. Clinically, ODZ1 is associated with decreased survival rates. Nevertheless, the association of the ODZ1-mediated invasion pathway with hypoxia, a major hallmark of GBM related to tumor invasion, has not been previously described. In this study, we evaluated expression of ODZ1 and its promoter methylation status under hypoxic conditions in vitro using glioma cell lines, and in vivo by administering pimonidazole (PIMO) to 14 GBM patients. Expression of ODZ1 mRNA was elevated in vitro and its protein expression in tumor samples was increased in regions staining positive for PIMO based on immunohistochemical analysis. *ODZ1*-promoter methylation status was assessed in DNA extracted from cells exposed to hypoxia and patient tumors using DNA extracted from PIMO-positive and –negative regions by immune-guided laser capture microdissection (LCM) and analyzed on the Illumina Human Methylation EPIC array platform. Hypoxic conditions led to a higher migratory capacity of GBM cells in-vitro, which correlated with an increase in ODZ1 mRNA level. A significant association between ODZ1-positivity and PIMO uptake (pODZ1-promoter was found to be hypomethylated in PIMO-positive regions compared to PIMO-negative regions, in addition to cells grown under hypoxia. Our data suggest that up-regulation of ODZ1 expression and ODZ1-mediated GBM invasion is associated with the hypoxic tumor microenvironment. It is likely that hypoxia leads to increased transcription of *ODZ1*mRNA through epigenetic regulation. ODZ1 expression within hypoxic tumor microenvironment may serve as a prognostic marker and therapeutic target in GBM.

GENE-06. THE ONCOHISTONE H3.3K27M DRIVES DIFFUSE INTRINSIC PONTINE GLIOMA INDEPENDENT OF FUNCTIONAL EZH2

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BACKGROUND: Diffuse Intrinsic Pontine Glioma (DIPG) is an incurable childhood brain tumor. Recent studies demonstrate that the replication independent oncohistone H3.3K27M, mutated in 65% of DIPG cases, inhibits Enhancer of zeste homolog 2 (EZH2), the enzymatic component of Polycomb Repressor Complex 2 (PRC2), leading to a global reduction in H3K27me3 levels and gene de-repression. Conflicting data exists supporting both an oncogenic and a tumor suppressor role for EZH2 in cancer. The present work addressed the functional significance of EZH2 and its cross-talk with the mutant histone H3.3K27M, in the context of DIPG pathogenesis. METHODS: Brainstem tumors were established by intracranial injections of NTv-a; $EZH2^{f/f}$neonatal pups using Replication Competent Avian Sarcoma leucosis virus long terminal repeat with splice acceptor (RCAS) viruses, expressing PDGF-B p53 shRNA, and RCAS-Cre/Y. Immunohistochemical staining (IHC) for Ki-67, H3K27me3, GFAP, Olig-2 and Nestin were performed on the Discovery ULTRA (Ventana). Cell proliferation assay (BrdU) was performed in neurosphere cultures established from brainstems of NTv-a; $EZH2^{f/f}$neonates, using the EZH2 inhibitor, EPZ011989 (1mM, Epizyme) for 5 days. RESULTS: *Ezh2* dele-

tion in NTv-a; *Ezh2*$^{f/f}$mice exacerbated DIPG pathogenesis, indicated by a 2.5-fold higher Ki-67 immunostaining (pEzh2deletion in neurospheres from Ntv-a; *Ezh2*$^{f/f}$neonates enhanced cell proliferation as did the addition of EPZ011989. Superimposition of the H3.3K27M mutant histone in this background, significantly shortened tumor latency, with a median survival of 54 days compared to 70 days in H3.3 wild type histone control group ($p<0.05$) and 50% incidence of Grade IV tumors. These results suggest that H3K27M may be oncogenic even in the absence of EZH2. FUTURE DIRECTIONS: Current studies aim to analyze the genetic and epigenetic landscape of these tumors using RNA-Seq and functional assays to determine whether EZH2 inhibitors will be a viable option for children with DIPG in the clinic.

GENE-07. GENOMIC ENHANCER METHYLATION IS ASSOCIATED WITH BIOLOGICAL AND CLINICAL FEATURES IN PITUITARY TUMORS

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The importance of enhancers has been recently highlighted in a pan-can transcriptomic analysis that revealed causal interaction between active enhancers and actionable genes as well as their prognostic value. DNA methylation is associated with enhancer activity and its interplay with the transcriptional machinery. However, pituitary tumors (PT) were not included in the pan-can study and the role of enhancer in these tumors is unknown. In order to address the methylation profile of causal (CausEnhs) and prognostic enhancers (PrognEnhs) described in the pan-can analysis, we analyzed the DNA methylome of two cohorts - an internal one comprised of 9 macroadenomas (6 nonfunctioning pituitary tumors-NFPT and 3 functioning - FPT; 5 invasive) and 5 non-tumoral pituitary specimens (NT) and an external cohort comprised of 24 PTs (18 NFPT + 6FPTs). Unsupervised analysis showed that PrognEnhs clustered according to PT functional status and were mainly hypermethylated in NFPT in relation to FPT, in both cohorts. PrognEnhs overlapped with differentially methylated enhancers (DME) that we previously identified in a supervised analysis comparing NFPTs and FPT from our cohort ($p < 0.03$). Among the 6758 PrognEnhs, 177 overlapped hypermethylated enhancers and 8 overlapped hypomethylated enhancers in NFPTs. Motif analysis of the ProgEnhs that overlapped hypermethylated enhancers identified a DNA signature including the core Basic Leucine Zipper Domain (bZIP domain), binding site for the transcription factors Fra-1/Fra-2,AP-1, Fosl2 and ATF3 (p-value < 0.0001), related to various oncogenic processes. In both cohorts, probes nearest or overlapping CausEnhs were mostly hypermethylated but not significantly associated with any clinical feature. These preliminary findings suggest that DNA methylation of enhancers associated with prognosis in other tumors may be involved in the functional status and oncogenic pathways in PT.

GENE-08. SCHWANNOMATOSIS SCHWANNOMAS HARBOR DISTINCT DNA METHYLATION PROFILES

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Schwannomas are characteristic manifestations of NF2 and schwannomatosis syndromes. However, the majority of schwannomas are solitary and sporadic. It is unclear whether and to what extent sporadic and syndrome-associated schwannomas or their histologic subtypes represent distinct biological groups. Clinically, although schwannomatosis schwannomas are considered benign, the majority of patients experience unmanageable pain; however, the underlying mechanism of this pain is not well understood. There is increasing evidence for DNA methylation profiling being able to distinguish biologically relevant tumor subgroups, even within the same cellular lineage and histopathologically similar tumors. In this study, we used Illumina Methylation EPIC arrays for methylome-based characterization of 88 schwannomatosis schwannomas, in comparison to 90 sporadic schwannomas and 14 NF-2 schwannomas. We performed unsupervised hierarchical clustering selecting 30,000 probes that showed the highest median absolute deviation (MAD) across all beta values. Three different clustering sets were utilized to obtain the most refined differentiation. Schwannomatosis schwannomas formed 3 distinct methylome-based subgroups, which were fully distinct from sporadic schwannomas and NF-2 schwannomas. Additionally, we performed copy number analysis using the DNA methylation data to infer gross chromosomal deletions or gains among the sporadic and syndromic schwannomas, in addition to schwannomas with hybrid features. Methylation subgroups were further correlated with clinical parameters including age, gender, anatomic location, tumor size, germline mutation status (LZTR1/SMARCB1), and 22q LOH, in addition to the histopathologic features associated with each tumor. Furthermore, RNA sequencing was performed to examine gene expression profiles associated with the 3 methylome subgroups and the data was integrated with DNA methylation profiles to establish the biological relevance of hypo- and hyper-methylation of the top varying CpG sites. Cumulatively, these data will be correlated with the extent and type of pain experienced by schwannomatosis patients to elucidate the underlying mechanism of pain.

GENE-09. FUNCTIONAL GENOMIC ELEMENTS DEFINED BY DNA METHYLATION CAN DISTINGUISH MENINGIOMA SUBGROUPS

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Despite advances in therapy, treatment of malignant meningioma remains challenging - the tumor usually progresses after treatment. Atypical and anaplastic (malignant) meningiomas (grades II/III) are high-risk meningiomas that are prone to recurrence and unfavorable prognosis. We seek to understand the mechanisms of meningioma progression after initial treatment. Recently, based on DNA methylation, two subgroups of meningioma were described with recurrence-free survival differences. Master regulators at distinct genomic elements define gene activity and tissue identity and deregulation have been shown to be involved in tumorigenesis. We hypothesize that functional genomic elements can contribute to meningioma progression/recurrence. Our aim is to use DNA methylation data to identify candidate noncoding elements and their connection with genes that might explain differences in meningioma prognostic subgroups. Using published DNA methylation markers that define favorable and unfavorable meningioma subgroups, we identified 1,330 differentially methylated probes ($p< 0.0001$, difference mean-methylation beta-value> 0.25) distributed across CpG islands (13%), shores (34%) and open-sea regions (53%). Focusing on probes within known functional genomics, we identified 18 highly conserved genomic enhancers that can potentially drive meningioma recurrence. We next investigated links between these enhancers and their targeted genes by incorporating GeneHancer annotation. We found that the unfavorable subgroup of meningiomas presented hypomethylation within enhancer regions that have the potential to target PARK7, ARID4B, and FBH1. ARID4B was previously shown to be highly active in high-grade meningiomas. Our preliminary results are the first to suggest that DNA methylation changes can be used to identify noncoding regions associated with meningioma prognosis. We will use additional independent data to confirm our preliminary results. Identification of noncoding regions associated with meningioma progression/recurrence will provide knowledge of the role of epigenomics in the development of malignant meningioma and of opportunities for targeted therapy.

GENE-10. IDENTIFICATION OF MXRA5 AND DSP AS RELEVANT TARGETS IN INFILTRATING ASTROCYTOMAS: A WHOLE EXOME ANALYSIS AT A SINGLE INSTITUTION

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BACKGROUND: Infiltrating astrocytomas (IA) comprise the most common primary brain tumors in adults. Herein we summarize our findings in an adult IA cohort enrolled as part of a precision medicine-based clinical trial at a single institution. DESIGN: We interrogated 85 IA samples over 81 distinct adults. Whole exome sequencing was performed and matched with peripheral blood samples for germline analysis. We present a summary of our findings and a comparison to commercially available approaches available in 68 (80%) of cases, in order to assess distinct approaches to the molecular characterization of IA. RESULTS: The most frequently altered genes detected by both WES and commercial platforms include *EGFR*, *CDKN2A/B*, *TP53*, *PTEN*, *NF1*, *IDH1, ATRX*, and *PIK3CA*. Certain alter-

ations such as *TERT*promoter mutations, which were present in the majority of IDH-wildtype IA's as detected by commercial platforms, were not assessed by WES. Several mutations in cancer related genes were revealed to be germline alterations in the WES pipeline alone, including in *ARID1A*, *BRCA1*, *NF1,* and *WT1*. In addition, several genes were noted to be recurrent at lower frequencies including CD209, DCHS2, DROSHA, FBN2, HECW2, MTUS2, OBSCN, PCLO, QRICH2, RBP3, SPTB, TENM3, VWF, MXRA5 and DSP. Notably, both MXRA5 and DSP were found to be associated with significantly differential survival when applied to the TCGA dataset for Low and High Grade Gliomas. CONCLUSION: The majority of the most common recurrent genetic alterations in the spectrum of IA are reliably detected by WES and at comparable rates to more targeted panels such as commercially available platforms; however exome sequencing pipelines require refinement to cover promoters such as TERT. Germline assessment is important to understand the implications of mutations in cancer related genes which are not currently reported by commercial platforms. Finally, mutations in DSP and MXRA5 may be potential therapeutic targets deserving of further study.

GENE-11. SMALL TERMINAL ALTERATIONS AND ALTERNATIVE LENGTHENING OF TELOMERES ARE A FEATURE OF IDH-MUTANT, 1p/19q NON-CODELETED GLIOMAS

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INTRODUCTION: Gliomas are typified by genetic variability and instability. The full potential of molecular genetics to classify tumors has not been realized. We previously proposed a novel glioma classification system that increases the sensitivity and specificity of diagnosis and improve prognostication. We have completed copy number array mutational analyses and a targeted next-generation sequencing 50-gene glioma panel on 148 Mayo Clinic formalin-fixed paraffin-embedded gliomas with subsequent data validation performed on 419 gliomas from the TCGA. RESULTS: Median age at resection was 49 years (range: 17–80; 95% CI: 46.5–51.6 years). There were 36 (24.3%) *IDH-mutant, 1p/19q codeleted* oligodendrogliomas; 46 (31.1%) *IDH-mutant, 1p/19q non-codeleted* astrocytomas and 66 (44.6%) *IDH-wildtype* gliomas. Small terminal alterations (STAs) defined as copy number variations (CNVs) IDH-mutant, 1p/19q non-codeleted tumors. Acquisition of $^{3}2$ STAs was observed in 47% IDH-mutant, 1p/19q non-codeleted versus 27% IDH-wildtype tumors ($P=1.8x10^{-5}$). In the TCGA dataset, these were seen in 67% versus 26% respectively ($P=2.8x10^{-16}$). STAs were glioma subclass-specific and did not correlate with overall genetic instability as determined manually or with a computational method. *ATRX* loss was observed in 27 of 41 (65.9%) tumors with an ATRX mutation versus 20 of 106 (18.9%) *ATRX-wildtype* tumors ($P=1.3x10^{-7}$). *ATRX* loss also correlated with the alternative lengthening of telomeres (ALT) phenotype and was seen in 25 of 31 (80.6%) ALT-positive versus 7 of 55 (12.7%) ALT-negative tumors ($P=1.5x10^{-9}$). STAs were present in 19 of 32 (59.4%) ALT-positive cases and 8 of 35 (22.9%) ALT-negative cases (P=0.0061). CONCLUSION: *IDH-mutant, 1p/19q non-codeleted* astrocytomas have a high prevalence of STAs. This is associated with *ATRX* loss and the ALT. These alterations do not appear to have *de novo* implications for survival but may provide novel diagnostic tools and therapeutic targets.

GENE-13. GENOMIC FUNCTIONAL ENHANCERS DEFINE POTENTIAL TUMORIGENESIS OF G-CIMP-LOW (IDH-MUTANT ASTROCYTOMA) TUMORS INDEPENDENT OF PROMOTER METHYLATION

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Glioma CpG-island methylator phenotype (G-CIMP) is associated with IDH mutation and presents better clinical outcomes than for IDH wildtype gliomas. However, a recent stratification of G-CIMP tumors identified a subgroup of patients with distinct epigenetic changes in intergenic regions linked to poor prognosis (G-CIMP-low) compared to G-CIMP-high. Several regulatory elements, such as enhancers and silencers, are located outside gene promoters and DNA methylation changes in these elements could play an important role in regulating the expression of distant genes. In order to identify potential active enhancers by loss of DNA methylation in G-CIMP-low, we investigated 1 G-CIMP-low, 2 G-CIMP-high and 2 non-tumor brain samples profiled by whole-genome bisulfite sequencing (methylome) and RNA-sequencing (transcriptome). Using the GeneHancer database, which includes known enhancers and their potential gene targets assessed by functional studies, we identified 109 downregulated and 856 upregulated genes in G-CIMP-low in relation to G-CIMP-high. These had no DNA methylation differences at their promoters between G-CIMP-low, G-CIMP-high and non-tumor brain samples. However, the associated candidate enhancers for these genes had distinct hyper or hypo-methylation of CpG specific to G-CIMP-low, compared to both G-CIMP-high and non-tumor brain samples. Downregulated genes are associated with negative regulation of both protein phosphorylation and immune response, such as PRKAG2, an AMP-activated protein kinase. G-CIMP-low tumors have expression levels similar to IDH wildtype glioblastomas whereas G-CIMP-high tumors are comparable to non-tumor brain samples. Upregulated genes are enriched for cell cycle and metabolism of RNA, which may relate to observed aggressiveness and proliferation in G-CIMP-low tumors. From this pilot data, we hypothesize that distal elements regulate the expression of genes in G-CIMP-low which leads to a more aggressive phenotype. These findings corroborate the importance of epigenetics in gliomas and propose that intergenic elements might be driving the G-CIMP-low tumorigenesis.

GENE-14. DNA METHYLATION AND PROTEOMIC ALTERATIONS IDENTIFY HISTOLOGICALLY-DEFINED TUMOR CELL POPULATIONS AND CHARACTERIZE INTRATUMOR HETEROGENEITY IN GLIOBLASTOMA

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BACKGROUND: Tumor heterogeneity presents a major challenge to cancer diagnosis and treatment. In addition to interpatient tumor variability, intratumoral heterogeneity characterized by distinct molecular and phenotypic profiles is increasingly recognized as a major cause of therapy resistance and cancer recurrence. Because DNA methylation patterns are largely responsible for determining cell-type-specific functioning, we hypothesized that distinct DNA methylation and proteomic alterations could be identified in histologically-defined invasive and proliferative tumor cell populations in human isocitrate dehydrogenase 1 (*IDH1*)-mutated and wild-type glioblastoma (GBM). METHODS: Formalin-fixed paraffin-embedded tissue sections of human adult *IDH1*-mutated and wild-type GBM were laser-microdissected (LM) into perinecrotic pseudopalisading tumor cells (PPCs), non-pseudopalisading tumor core cells (NPPCs), invasive subpial spread (SPS) and perivascular satellitosis tumor cells and brain adjacent to tumor cells prior to analysis and compared to non-microdissected tumor (NMT) and/or germline DNA. Genome-wide DNA methylation and chromosomal copy numbers were determined with Infinium MethylationEPIC 850K BeadChip and intratumoral DNA methylation patterns compared by unsupervised hierarchical clustering. Label-free quantitative liquid chromatography-mass spectrometry of proteins was performed and proteins differentially expressed across LM areas subjected to pathway enrichment analysis. RESULTS: Unsupervised hierarchical classification of DNA methylation patterns for each LM area and NMT demonstrated remarkable clustering for all patients, based on methylation probe and methylated gene patterns. Proteomics analysis showed upregulation of hypoxia-inducible factor-1 inducible proteins in hypoxic PPCs. Out of 1819 proteins quantified, 5 were overexpressed and 9 underexpressed more than 10-fold in SPS compared with NPPCs and associated with alterations in metabolism, transport, extracellular matrix and apoptosis. Correlation of protein expression and DNA methylation patterns was noted. CONCLUSIONS: Compared to NPPCs, SPS cells migrating toward the invasive edge share a relatively consistent epigenetic and proteomic signature, suggesting potentially targetable common mechanism(s) of invasion shared among GBM.

GENE-15. CELL AUTONOMOUS MECHANISMS OF SEX DIFFERENCES IN RESPONSE TO TUMOR SUPPRESSOR LOSS

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Sex differences in incidence and prognosis are observed for nearly all human diseases, including brain tumors. Males are more likely to develop brain tumors – a phenomenon observed throughout the world and across all ages. Sex differences in brain tumor incidence suggest there may be intrinsic differences in how male and female cells respond to oncogenic stress. Cell autonomous sex differences can arise from one of two mecha-

nisms: differences in sex chromosome complement or organizational effects of gonadal hormones. To investigate whether there are sex differences in the cellular response to tumor suppressor loss and whether sex chromosome genes or organizational effects of gonadal hormones are responsible, we utilized the four core genotypes (FCG) mouse model. In the FCG model, the testis-determining gene, Sry, is removed from the Y-chromosome and inserted onto an autosome. This results in four genotypes, XY/Sry+ (XY with testes), XY/Sry- (XY with ovaries), XX/Sry+ (XX with testes), and XX/Sry- (XX with ovaries), and allows for the separation of gonadal and chromosomal sex effects. Using astrocytes harvested from FCG pups, we introduced loss of function of the tumor suppressor p53, the most commonly mutated gene in cancer, and then assessed cell proliferation. Following loss of p53 function, astrocytes from mice with testes had greater rates of cell proliferation than those from mice with ovaries, regardless of chromosomal sex (p=0.028). These results suggest that organizational effects of gonadal hormones may underlie sex differences in brain tumor incidence. Since organizational effects are established through epigenetic marks, this identifies sex differences in the epigenetic landscape as an important mediator of sex differences in brain tumor incidence, and highlights the need for sex specific analyses in clinical trials of epigenetic therapeutics.

GENE-16. CLINICALLY AGGRESSIVE MENINGIOMAS ARE CHARACTERIZED BY MUTATIONAL SIGNATURES ASSOCIATED WITH DEFECTIVE DNA REPAIR AND MUTATIONS IN CHROMATIN REMODELING GENES

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BACKGROUND: Up to 20% of meningiomas are aggressive tumors with high recurrence rates and poor prognosis. Biomarkers predicting the risk of an unfavorable clinical course are lacking although aberrations in *NF2*, increased copy number variations and a hypomethylated phenotype have been associated with more aggressive behavior. Mutational signatures (MS) are characteristic patterns of somatic mutations seen in cancer genomes associated with aging, exposure to certain mutagens, or defective DNA repair. We aimed to identify MS patterns in clinically aggressive meningiomas. METHODS: We performed whole exome sequencing of 18 *de novo* meningiomas (locally invasive and recurrent WHO I, n=6; atypical WHO II, n=4; anaplastic WHO III, n=8). Median PFS was 18.9 months. Copy numbers and DNA methylation phenotype were assessed by DNA methylation array analysis. Mutational signatures were identified using published signature algorithms (COSMIC). RESULTS: MS1 and MS5 (aging) were found in 18 (100%) cases. MS associated with defective DNA MMR were highly prevalent: MS20 and MS26 were detected in 18 (100%) and MS6 in 2 (12%) cases. MS12 (unknown etiology) was present in 14 (82%) cases. Despite the association with defective DNA MMR, none (0%) of the MS6 cases harbored somatic mutations associated with DNA MMR while MS12 tumors were enriched for mutations in DNA MMR (43%), chromatin remodeling (36%) and other cancer-associated genes (7%). MS6 tumors had significantly lower indels compared to non-MS6 tumors (p=0.01). Tumors with mutations in chromatin remodeling genes had a significantly higher rate of single nucleotide variants (SNVs) compared to cases without such mutations (p=0.02). CONCLUSIONS: MS associated with defective DNA MMR were highly prevalent in this set of aggressive meningiomas. However, despite the association with DNA MMR, MS6 meningiomas harbored no somatic mutations associated with DNA MMR while MS12 tumors were enriched for mutations in DNA MMR, chromatin remodeling and cancer-associated genes.

GENE-17. TOP2B REGULATES CDK4 SPLICE VARIANTS IN GLIOMAS

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Topoisomerase IIB (TOP2B) is known to decoil and decatenate DNA, releasing chromatin torsional forces. Whereas TOP2B has been implicated in transcript splicing, direct evidence supporting this function, through localization of TOP2B to specific chromatin sites and in association with specific types of cancer, is modest at best. Here, we report our preliminary activities that are intended to address this knowledge gap. GSEA differential expression analysis using TCGA glioblastoma data showed that high TOP2B expression is associated with elevated expression of cell cycle-related genes (p-value= $1.67E^{-05}$). To investigate the possible association of TOP2B with specific gene sequences we used TOP2B ChIP-seq in analyzing glioma cell lines TS543 and BT142, and found TOP2B associations with the introns of especially long genes (gen length ≥ 100Kb, p-value= 0.001). Functional annotation analysis for genes with TOP2B intron binding showed a significant enrichment for genes with multiple known splice variants (p-value= $1.9E^{-11}$). To determine effects of TOP2B inhibition on splice variant expression, we treated BT142 cells with the TOP2 inhibitor ICRF-193 and examined treatment effects on splice variant expression using paired-end RNA-seq. We observed altered splicing of 319 genes following TOP2 inhibition, with increased expression of splice variant CDK4-009 being the most significant of all splice variant expression changes (p-value= $1E^{-54}$). This result corresponded well with TCGA data whose analysis showed a strong positive correlation between TOP2B and CDK4-009 expression (rho= -0.3 and p-value= $7.28E^{-05}$). CONCLUSIONS: Our results support TOP2B expression and activity as being influential in regulating the levels of CDK4 splice variant 009 in GBM.

GENE-18. DIVERGENT CLONAL EVOLUTION OF MELANOMA BRAIN METASTASES DURING TREATMENT WITH IMMUNOTHERAPY

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Reversal of immune cell exhaustion through immune checkpoint blockade (ICB) has become a first-line approach for patients with metastatic melanoma due to success in controlling extracranial tumors. However, patients frequently experience discordant responses, with extracranial response and intracranial progression. We hypothesize that ICB exerts selective pressure leading to the clonal evolution of treatment resistant clones that ultimately culminate in disease progression. We collected a cohort of 97 patients, encompassing 312 pre- and post-immunotherapy melanoma tumors, for whole exome sequencing (WES) and included primary, extracranial, or intracranial samples. Each tumor was analyzed for somatic mutations, copy number alterations, neoantigen profile, and patient specific phylogenetic trees were constructed encompassing a tumor's genetic subclones. Heterogeneity of the tumor microenvironment was evaluated using high multiplicity single-cell immunofluorescent staining (CycIF). Single cell sequencing was performed on fresh tissue from 4 pre-treatment and 14 post-immunotherapy melanoma brain metastases using the Smart-Seq2 protocol. WES of pre- and post-immunotherapy tumors yielded distinct patterns of clonal evolution and immunoediting within brain metastases compared to their extracranial counterparts, including mutations in *B2M*. In paired pre- and post-immunotherapy samples, CycIF demonstrated decreased in CD8 infiltration and increased CD45RO, FOXP3, and PD-L1 staining suggesting less cytotoxic, terminally differentiated T cells in resistant tumors. Single cell sequencing analysis of 3,974 tumor and immune cells demonstrated patient-specific tumor clustering and gene expression profiles mediating resistance to ICB. In conclusion, we document, for the first time, evidence of ongoing

branched evolution during immunotherapy in brain metastases with divergence compared to systemic sites of disease. Next-generation sequencing provides novel insights into clonal evolution mediating discordant responses of intra- and extracranial sites and immunosuppressive features of the intracranial tumor microenvironment. Targeting these mechanisms of resistance provide potential therapeutic avenues for patients with progressive intracranial disease.

GENE-19. GAINING A BETTER UNDERSTANDING OF DNA METHYLATION FEATURES ASSOCIATED WITH SEX DIFFERENCES IN GLIOBLASTOMA

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Glioblastoma displays strong sexual dimorphism with male having an increased prevalence and poorer prognosis. While sex based methylation differences in the MGMT promoter have been described in GBM patients, we expanded on these findings with a full genome interrogation of sex-based DNA methylation differences in GBM patients (n = 56 females, n = 77 males). Using Illumina 450k DNA methylation data from The Cancer Genome Atlas (TCGA), we found 359 probes and 12 regions significantly differentially methylated between males and females (sex chromosomes were excluded from the analysis). Males had three times the number of probes significantly differentially hypermethylated than females. In males hypermethylated probes occurred in known enhancer regions at a rate of 4:1 as compared to females. Areas of hypermethylation in females predominately (65%) occurred in CpG islands. An analysis of DNA motif binding sites showed multiple zinc finger transcription factor binding sites located in genomic regions hypermethylated males. Binding sites of KLF6, an important tumor suppressor known to increase p21 expression through a p53 independent pathway, were located in areas significantly hypermethylated in males. We established significant correlation between expression of p21 and methylation of KLF6 binding sites. These findings provide a potential biological basis for our previous observation of increased p21 activity and cell cycle arrest in response to DNA damage in female, but not male GBM astrocytes. Additionally, we found that NFAT5 transcription factor binding sites were significantly hypermethylated in females. These results point to possible protective biology in females, where repression of NFAT5 results in reduced integrin-induced cell dispersion and increased p21 expression results in decreased proliferation. Understanding the molecular basis for sex-based differences will improve our understanding of tumor biology and pave the way for novel diagnostics and a personalized approach for the development of more effective therapies.

GENE-20. A NOVEL K-M ENHANCER REGULATES TEMOZOLOMIDE RESISTANCE AND TUMOR GROWTH IN GLIOBLASTOMA

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Emergence of temozolomide (TMZ) resistance and increased tumor aggressiveness are common in recurrent glioblastoma. However, mechanisms promoting these phenomena are incompletely understood. Previously, we described *in vivo* models of acquired TMZ resistance in GBM patient derived xenografts (PDXs), including MGMT expressing TMZ resistant subline (GBM12-3080) and TMZ naïve subline (GBM12-5199). To identify mechanisms associated with MGMT upregulation, the epigenetic differences between these lines were analyzed by ChIP-seq. Compared to GBM12-5199, GBM12-3080 displays H3K4me3 within the MGMT promoter region and H3K36me3 within the MGMT gene body region, indicative of an active epigenetic landscape. In addition, a genomic region localized between the promoters of *MKI67*, which encodes Ki-67 protein, and *MGMT* acquired active enhancer marks (H3K4me1 and H3K27ac) in GBM12-3080. Similar histone marks increase within this putative enhancer region were identified in paired samples from TMZ naïve and recurrent tumors in three out of eight PDX models and one out of three recurrent patient tumors. Using H3K27ac occupancy as a guide, tiled fragments of the putative enhancer were cloned into a luciferase reporter system, which ultimately identified a 1.5 kb region with robust enhancer activity. In parallel, a chromatin conformation capture assay demonstrated physical interaction between this 1.5 kb enhancer region and the MGMT promoter, which was only detectable in GBM12-3080. Finally, deletion of this enhancer by CRISPR/Cas9 in both GBM12-3080 and MGMT expressing glioblastoma cell line (SKMG3) reduced MGMT expression by greater than 90% and reduced the TMZ IC_{50} by 71% and 97%, respectively. Consistently, the expression of the adjacent gene, *MKI67*, was reduced by approximately 40% in both lines, and tumorigenicity of GBM12-3080 deletion clones was markedly suppressed. In conclusion, K-M enhancer regulates MGMT and Ki-67 expression independent of DNA methylation. Inhibition of this enhancer may sensitize tumors to TMZ and reduce tumor growth in a sub-population of recurrent glioblastoma.

GENE-21. A COMMON FETAL DEVELOPMENTAL ORIGIN FOR PFA EPENDYMOMA, PFB EPENDYMOMA, AND CEREBELLAR PILOCYTIC ASTROCYTOMAS?

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Single cell RNA-sequencing (scRNAseq) of murine cerebellum at nine fetal and immediate post-natal (E10-P14) times points on >60,000 individual cells to identified >30 transcriptionally distinct cell clusters. Based on marker gene expression, many clusters resemble known cerebellar stem, progenitor, and differentiated cell types, while other clusters are more novel. Pseudo-time trajectory assisted in the reconstruction of known and novel developmental lineages, including the lineage of the cerebellar radial glia. A population of stem cells in the ventricular zone (VZ) gives rise to the progenitors of the GABAergic cerebellar interneurons, as well as the gliogenic progenitor cells, which subsequently become Bergmann glia and astrocytes. A novel, but clearly distinct and robust cluster of cells with transcriptional similarity to both the roof plate and the rhombic lip was identified. Comparison of bulk RNA-seq from human PFA, PFB, and cerebellar pilocytic astrocytomas (C-PA) reveals that all three-tumor types best transcriptionally match the gliogenic progenitor cells, with some similarity to VZ stem cells and the 'roof plate like' stem cells. Furthermore, all three tumor types are transcriptionally much more similar to the gliogenic progenitors at E16 than at E14 or E18. Subclustering of gliogenic progenitors reveals significant intra-cluster heterogeneity, with the ependymomas transcriptionally matching one subcluster, and the C-PA clearly matching a very different subcluster. scRNAseq of human PFA and PFB ependymomas reveals multiple tumor cell clusters within a given human ependymoma, with some clusters matching most closely to the gliogenic progenitors, and others matching best to the 'roof plate like' stem cells. Similarity to the 'roof plate stem cells' (E10-E14), and gliogenic progenitors (E14-E18) suggests an embryonic origin for PFA, PFB, and C-PA, suggests specific novel cells of origin, and offers a novel opportunity to understand posterior fossa tumor transcriptomic targets for novel therapy.

GENE-22. RE-PROGRAMING CHROMATIN WITH A BIFUNCTIONAL LSD1/HDAC INHIBITOR INDUCES THERAPEUTIC DIFFERENTIATION IN DIPG

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Diffuse intrinsic high grade glioma (DIPG) is an almost universally fatal tumor of childhood characterized by epigenetic dysregulation driven by somatic H3.3/H3.1 K27M mutations observed in >80% of tumors. We conducted a chromatin-focused CRISPR screen and identified a novel strategy to inhibit the growth of patient-derived DIPG cells by co-targeting lysine specific demethylase 1 (LSD1) and histone deacetylases (HDACs). Consistent with the genetic data, we demonstrate that a bifunctional

inhibitor of HDACs and LSD1, Corin, inhibits the growth of DIPG cells both *in vitro* and in orthotopic xenografts. Mechanistically, co-targeting LSD1 and HDACs with Corin synergistically alters the levels of histone modifications in DIPG, rescuing H3K27me3 levels suppressed by the dominant negative effects of K27M mutant histones and inducing simultaneous increases in both HDAC-targeted H3K27ac and LSD1-targeted H3K4me1 at thousands of genomic locations. Coincident with these chromatin changes, we observe robust transcriptional changes in DIPG cells, including the repression of cell cycle-related genes and the activation of neuronal differentiation genes. Consistently, phenotypic assays reveal that Corin reduces S-phase and Ki67+ proliferating cells, induces a neuronal-like morphology, alters the expression of stem and differentiation markers, and increases DIPG cell death. Finally, analyses of patient DIPG expression datasets indicate that Corin-dependent transcriptional signatures are overrepresented in normal brain compared to DIPG tumors and correlate with increased patient survival time. Together, these data reveal that co-inhibiting LSD1 and HDACs synergistically reduces DIPG growth by reprogramming the chromatin landscape to activate a latent differentiation response, and suggest a strategy of co-inhibiting LSD1 and HDACs with Corin to treat DIPG.

GENE-23. PREVIOUSLY IDENTIFIED COMMON GLIOMA RISK SNPs ARE ASSOCIATED WITH FAMILIAL GLIOMA

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BACKGROUND: Approximately 5% of gliomas occur in individuals with a family history of glioma, and first-degree relatives of brain tumor cases have a two-fold increase in risk of brain tumor. Family-based studies have had little success in identifying high penetrance risk variants. Recent somatic characterization has shown that tumors from familial cases are indistinguishable from sporadic cases, suggesting that familial cases may arise through similar mechanisms of gliomagenesis, and therefore may be associated with common variants as well as rare mutations. In this analysis, we assessed whether previously identified common risk variants are associated with familial glioma. METHODS: Data were obtained from the Glioma International Case Control (GICC) Study for 447 familial cases and 3,286 controls. We assessed 25 risk loci previously identified by glioma GWAS, and odds ratios (OR) and 95% confidence intervals (95%CI) were calculated using an additive genetic logistic regression model adjusted for age, sex, and the first principal component. Results were considered significant at p TERT, *EGFR*, *CCDC26*, *CDKN2B*, *TP53*, and *RTEL1*. The strongest association was at rs55705857 (*CCDC26*, OR=2.5, $p=1.14\times10^{-14}$). These SNPs were further examined using a case-only approach comparing familial to non-familial cases, and there was no significant difference in allele frequencies by family history status. CONCLUSIONS: In this analysis we identified a significant association between familial glioma and six common risk variants previously identified by glioma GWAS. This provides further evidence of shared pathways of genetic risk and gliomagenesis between familial and non-familial glioma. Further exploration is necessary to determine the overall contribution of common genetic variation to risk of familial glioma.

GENE-24. IMPACT OF THERAPY ON EVOLUTION OF GBM

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Glioblastoma multiforme (GBM) is the most malignant and common primary brain cancer in adults, with a 2.5-year survival of only ~8%. In this single case study we have analysed the development of a primary GBM over four years. We performed WGS and RNAseq on three, spatially distinct samples of the treatment naïve primary tumour, the first recurrence and, the fatal second recurrence. The DNA analysis showed only one shared mutation in all of the 9 samples: a mutation in EGFR (G598V). We hypothesise that subsequent tumours developed from an original clone bearing this mutation. This mutated oncogene is located on an extrachromosomal circular DNA structure known as double minute (DM), which is found in an estimated 40% of GBM. We performed long-range sequencing using the 10X genomics technology to reconstruct the DM and shed light on its emergence. We observed a massive increase in mutations from the first to the second recurrence, showing predominantly C > T/ A > G transitions, which is a pattern typical of TMZ treatment. The fact that the hypermutation did not appear until the second recurrence raises questions about the mechanism of hypermutation. The therapeutic apoptotic action of TMZ relies on the function of the mismatch repair (MMR) pathway. In the third recurrence we observed disruptive mutations of MSH2, 5 and 6 all of which are crucial to the MMR pathway. The pattern of mutations of these genes varied between the samples taken from the second recurrence, however, all three samples showed hypermutation. This suggests that the hypermutation observed after TMZ treatment only occurs if the MMR is damaged, but that this damage does not need to be at a specific locus. Using the CRISPR-CAS9 technology we are currently exploring the impact of MMR mutations on patient derived GBM cell lines.

GENE-25. LOSS OF m^{6}A RNA METHYLATION DURING GLIOMA STEM CELL DIFFERENTIATION IS REGULATED BY MIRNAS AND PROMOTES TRANSLATION EFFICIENCY

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The dynamic and reversible N^6-methyladenoside (m^{6}A) affects eukaryotic mRNA localization, stability, splicing and translation. However, the role of m^{6}A RNA methylation in the regulation of translation efficiency in human cancer cells is largely unknown. Here, using ribosome profiling, integrated transcriptome and m^{6}A RNA sequencing analyses, we determined that as patient derived glioma cells transition from stem cells to differentiated cells, they exhibit loss of m^{6}A RNA methylation and an increased rate of translation of the demethylated transcripts. We identified that the sequence motifs of the m^{6}A peak regions of genes that lose m^{6}A RNA during glioma stem cell differentiation, are complementary to the seed sequences of specific miRNAs. Expression of these target specific miRNAs results in loss of m^{6}A RNA methylation without affecting transcript levels. Moreover, miRNAs induce cellular demethylase activity, increase the association of FTO with RNA in nuclear speckles and the binding of FTO to the miRNA-targeted and demethylated transcripts. This study highlights a critical role of m^{6}A RNA methylation in regulation of translation in human cancer cells and identifies regulatory miRNAs that functionally influence the epitranscriptome during glioma stem cell differentiation.

GENE-26. MOLECULAR CHARACTERIZATION OF BENIGN AND MALIGNANT PERIPHERAL NERVE SHEATH TUMORS THAT OCCUR IN SPORADIC AND SYNDROMIC SETTINGS

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INTRODUCTION: Neurofibromas, a peripheral nerve sheath tumor commonly associated with Neurofibromatosis Type 1, are broadly catego-

rized as cutaneous or peripheral nerve neurofibromas (localized or plexiform lesions). More recently, atypical neurofibromatous neoplasm of unknown biological potential (ANNUBP) has been described and believed to be a premalignant tumor, although the oncogenic drivers of malignant transformation are poorly understood. In this study, we establish the spectrum of genomic drivers of neuronal tumors, benign and malignant, in sporadic and syndromic settings. METHODS: We performed multiplatform genomic analysis of a total of 110 cutaneous neurofibromas, peripheral nerve neurofibromas and malignant peripheral nerve sheath tumors (MPNSTs). We performed methylation profiling and RNA sequencing on these tumors. Genomic data was bioinformatically analyzed to identify biologically relevant subgroups. Correlation and validation of key drivers were carried out using a series of IHC and in-vitro studies. RESULTS: Consensus clustering of methylation data reliably distinguished between cutaneous and peripheral nerve tumors that supports the theory that cutaneous neurofibromas have a distinct cell of origin. Copy number analysis (CNA) identified a loss of 9p in 35% of peripheral nerve neurofibromas. Consensus clustering of peripheral nerve neurofibromas alone identified 3 subgroups, with group 1 tumors having no CNAs and groups 2&3 enriched for tumors with CDKN2A loss ($p < 0.05$). In addition, ANNUBP were associated with the loss of CDKN2A ($p < 0.05$). Neurofibromas with loss of CDKN2A had gene sets associated with H3K27me3 and sarcomas upregulated, while genes associated with neuronal and Schwann cell signature downregulated. CONCLUSION: The genomic and epigenomic landscape of neurofibromas is poorly understood. We identified that atypical neurofibromas have loss of CDKN2A, suggesting that it is lost early in malignant transformation. The loss of CDKN2A may lead to dysregulation of H3K27me3, and further work is needed to identify the molecular alterations and pathways involved in malignant transformation.

GENE-27. GENOME-WIDE DNA METHYLATION PROFILING IN GRADE II AND III GLIOMAS REVEALS A SUBSET OF GENES WITH PROGNOSTIC SIGNIFICANCE CONTROLLED BY PROMOTER METHYLATION

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This study sought to identify promoter-CpG methylation sites that are significantly correlated with overall survival and gene expression regulations in grade II/III gliomas. Fifty-six LGG specimens collected from the University Medical Center Freiburg were profiled using the Methylation 450K array, in a discovery cohort, which was followed by analysis of 471 LGG cases from The Cancer Genome Atlas (TCGA) in a validation cohort. Association between each CpG probe and patient overall survival (OS) was assessed in a Cox model with IDH1/2 mutations using false discovery rate (FDR) < 0.05. Probes were selected based on training association (FDR < 0.05) and being located on promoter regions. Genes were selected based on their correlation of expression and methylation (TCGA dataset only). The prognostic values of the selected genes were determined using univariable Cox model in the TCGA LGG cohort. The correlation of CpG-methylation and gene expression was determined using Pearson Correlation method. 40% of total CpG probes from the Freiburg cohort were found significantly associated with OS of LGG patients in the univariable Cox model, and 53970 CpG probes were validated in the TCGA cohort. 18.3% of 53970 probes are located on promoter regions. 99.7% of transcription start site (TSS) probes show that high promoter methylation levels predict better OS. After incorporating TCGA RNA seq dataset, 2145 CpG probes and 1331 genes were identified, which associate with OS. There was no probe significantly associated with OS in multivariable Cox model with IDH mutation status as co-variate. We identified genes whose promoter methylation regulate their expressions and are associated with favorable LGG patient outcome by controlling cancer cell movement, death and survival, and proliferation. Further studies are underway to identify select genes as therapeutic targets in LGGs, employing both in vitro and in vivo preclinical models.

GENE-28. METHYLOMES AND TRANSCRIPTOMES VARY ACROSS IDH1 MUTANT CANCERS

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Since their discovery in gliomas, mutations in isocitrate dehydrogenases 1 and 2 (collectively referred to as "IDH1^{mut}") have been discovered in a variety of cancers, including acute myeloid leukemia (AML), melanoma, and cholangiocarcinoma. While IDH1^{mut} promotes genomic hypermethylation in all these cancers, glioma remains the only tumor in which IDH1^{mut} is a consistently favorable prognostic marker, for reasons that are unclear. We therefore hypothesized that the pattern of DNA methylation, the resultant transcriptomic profiles, and specific genes suppressed, would all vary among IDH1^{mut} cancers according to tissue of origin. We analyzed Illumina 450K and RNA-Seq data from The Cancer Genome Atlas, including WHO grade II-IV gliomas (N=647; 427 IDH1^{mut}, 220 IDH1^{wt}), AML (N=194; 15 IDH1^{mut}, 179 IDH1^{wt}), melanoma (N=475; 23 IDH1^{mut}, 452 IDH1^{wt}), and cholangiocarcinoma (N=45; 7 IDH1^{mut}, 38 IDH1^{wt}). Using the tcgaWorkflow R package, we compared CpG methylation using Wilcoxon tests on beta values and transcriptomic read counts using negative binomial generalized log-linear models for IDH1^{mut} versus IDH1^{wt} tumors. A CpG site was considered hypermethylated if its mean beta value in IDH1^{mut} cancer was >0.15 relative to the matching IDH1^{wt} cancer. P-values were false discovery rate (FDR)-corrected. Of 264,735 analyzed CpG sites, 70,591 (19%) were hypermethylated in IDH1^{mut} gliomas compared to IDH1^{wt} gliomas. In contrast, only 3%, 2%, and 4% of CpG sites were hypermethylated in IDH1^{mut} AML, melanoma, and cholangiocarcinoma, relative to each of their IDH1^{wt} counterparts. Methylation-associated transcriptome differences were also more pronounced in IDH1^{mut} gliomas. Key promalignant genes that were uniquely hypermethylated and downregulated in IDH1^{mut} gliomas, relative to other IDH1^{mut} cancers, included *ERBB2* (HER2), *LGALS1* (galectin-1), and *PDPN* (podoplanin), among others. These data suggest that the extent and targets of IDH1^{mut}-induced genomic hypermethylation vary greatly according to the cellular context, and may help explain why IDH1^{mut} is only a favorable prognostic marker in gliomas.

GENE-29. DLL3 AND ETV1 ARE INACTIVATED/METHYLATED IN CIC WILD-TYPE, IDH-MUTATED, 1p/19q-CODELETED GLIOMA

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Adult diffuse glioma that harbor *IDH* mutation and 1p/19q codeletion commonly have *CIC* mutations; however, the functional and prognostic significance of *CIC* mutations remains unclear. We hypothesized that when *CIC* is not mutated, other genes may be inactivated by hypermethylation. Identifying such genes may help to further understand the biology of 1p/19q-codeleted glioma. To test our hypothesis, we utilized a two-stage design. In the discovery stage, 72 *IDH*-mutated 1p/19q-codeleted tumors were utilized from TCGA (40 *CIC* mutated, 32 wild-type); all via Illumina 450K methylation array. In the validation stage, 43 *IDH*-mutated 1p/19q-codeleted tumors were utilized from Mayo Clinic (33 *CIC* mutated, 10 wild-type); all via Illumina 850K methylation array. All tumors also had *TERT* promoter mutation. Genome-wide significance ($p \leq 5 \times 10^{-8}$) was used in the discovery stage to identify differentially methylated probes between *CIC* mutated vs wild-type tumors. Seven probes reached significance in TCGA; four validated at pCIC. *DLL3*, located on 19q, had a mean change in beta value between *CIC* wild-type and mutated tumors of 0.28 ($p=1.29 \times 10^{-8}$) and 0.24 (p=0.0024) in TCGA and Mayo, respectively. *ETV1*, located on 7p, had a mean change in beta value of 0.30 ($p=1.37 \times 10^{-8}$) and 0.15 (p=0.00011) in TCGA and Mayo, respectively. In the 72 TCGA tumors, we observed an inverse correlation between *DLL3* methylation and gene expression amongst *CIC* mutated and wild-type tumors; -0.42 (p=0.0063) and -0.33 (p=0.069), respectively. There was also an inverse correlation between *ETV1* methylation and gene expression amongst *CIC* mutated and wild-type tumors; -0.68 (pDLL3 and *ETV1* was downregulated in *CIC* wild-type vs mutated tumors (p=0.000027 and 0.0000028, respectively). We confirm that *ETV1* has higher gene expression in *CIC* mutated vs wild-type tumors. Our results suggest that *DLL3* expression is inactivated by promoter methylation in *CIC* wild-type 1p/19q-codeleted oligodendrogliomas; thus, *DLL3* may be an alternative target gene on 19q.

GENE-30. INCREASED TUMOR MUTATIONAL LOAD AFTER RADIOTHERAPY AND TEMOZOLOMIDE IN PROGRESSING GLIOBLASTOMA A PROSPECTIVE STUDY

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BACKGROUND: Tumor mutational load (TML) is the number of nonsynonymous mutations in a tumor sample. TML has proved predictive of response to immune therapy (IT) in other TML-high tumors. Research in

TML in glioblastoma (GBM) is limited and has to our knowledge not been done prospectively in paired samples before and after treatment. MATERIALS AND METHODS: Over a 2-year period from February 2016 to March 2018, 27 patients with newly diagnosed GBM were included. All patients had one biopsy at diagnosis and another when having secondary surgery due to progression. Three patients had three surgeries. We noted clinical and pathological data. Blood samples were extracted and whole exome sequencing has been performed. We are now investigating TML including clonal and subclonal mutations, mutations in mismatch repair genes and the aberrant cell fraction. TML will be reported as per Megabase exome and will be adjusted for coverage in each sample. RESULTS: All the included patients had primary GBM with isocitrate dehydrogenase (*IDH*) wild type. *O*-6-methylguanine-DNA methyltransferase (MGMT) was methylated in 6 patients (22%) and the majority of patients have been treated with chemo/radiation with Temozolomide. Since the patients were included from our daily clinical oncological department, the clinical characteristics resembled the average GBM patient according to age, performance status, treatment, progression-free survival and overall survival. CONCLUSION: Analyses are forthgoing and will be ready at the time of the SNO-meeting. An agreement of how to report TML is strongly needed and TML should be included in future trials in GBM.

GENE-31. MULTIPLATFORM MOLECULAR PROFILING AND QUANTITATIVE IMAGING OF AN ANAPLASTIC EPENDYMOMA REVEALS INTRATUMORAL HETEROGENEITY

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OBJECTIVES: Ependymomas are associated with distinct anatomic, genomic and clinical characteristics, but the diversity of molecular features within individual ependymomas is incompletely understood. Here, we perform multiplatform molecular profiling from 6 spatially- and radiographically-distinct regions within a single anaplastic ependymoma to shed light on intratumoral heterogeneity. METHODS: Immunohistochemistry, UCSF500 sequencing, whole exome sequencing, 850k DNA-methylation profiling and RNA-sequencing were performed to define the genomic, epigenomic and transcriptomic characteristics of stereotactically-collected samples that were separated by an average 19 mm (range 6–34 mm). Data were analyzed using hierarchical clustering, principal component analysis, molecular phylogenetic approaches, and multi-parametric radiologic techniques. RESULTS: Immunohistochemistry revealed diffuse L1CAM positivity, and UCSF500 detected chromosome 11 chromothripsis, suggestive of *C11orf95-RELA*fusion. All 6 regions expressed equivalent ependymoma markers such as *L1CAM*and *CCND1* byRNA-sequencing, which also confirmed *C11orf95-RELA*fusion. Principal component analysis of transcriptomic data identified three transcriptionally distinct tumor regions that were delineated by stem/proliferative genes (*HOXB3*, *OLIG2*, *PTPRN2*), neuronal differentiation genes (*DLK1*, *LBX1*, *HDAC9*) or immune/stress response genes (*CXCL8*, *HSPA1B*, *VEGFA*). DNA methylation analysis was consistent with *C11orf95-RELA*fusion ependymoma, but also identified three epigenetic profiles with similar characteristics to those discovered by RNA-sequencing from different tumor regions. Whole exome sequencing identified shared *SETD2*and *IL5RA*mutations throughout the tumor, as well as private mutations specific to individual regions, including *MUC4*, *ZNRF3* and *TGFBR2*. Stem/proliferative regions were additionally characterized by *SETD2*deletion and increased cerebral blood flow relative to other regions by magnetic resonance perfusion. CONCLUSION: Anaplastic ependymoma is associated with a previously unappreciated molecular heterogeneity that may influence tumorigenesis and design of molecular therapeutics.

GENE-32. ASSOCIATION OF OLIGODENDROGLIAL MORPHOLOGY, MOLECULAR FACTORS AND OVERALL SURVIVAL IN PATIENTS WITH LOWER GRADE GLIOMAS

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Presence or absence of 1p/19q codeletion is more closely associated with clinical outcome as well as the biology of tumor than histological diagnosis in lower grade gliomas (LrGGs). Nonetheless, it is still controversial if classic oligodendroglial morphology might be relevant to patients' prognoses. The subjects were institutionally-diagnosed grade II-III gliomas resected at Keio University Hospital from 1990 through 2016. Biopsy cases were excluded. The tumors were reassessed according to WHO 2007 classification. Presence or absence of the classic oligodendroglial features (classic for oligodendroglioma, CFO) was also assessed. The association between either pure oligodendroglioma diagnosis or presence of CFO and patients' survival or any of the molecular factors including copy number aberration (CNA) status, IDH mutations, ATRX mutations, and TERT mutation were assessed. 94 LrGGs were included in the study. There was only 36% concordance between the original institutional histological diagnosis and the reassessment. In the IDHmut/Codel tumors, there was no molecular factors that was associated with pure oligodendroglial diagnosis, CFO status or overall survival (OS). Interestingly, we found longer OS in pure oligodendroglial diagnosis (p= 0.023). In the IDHmut/Noncodel tumors, some molecular factors (ATRXmut, TERTmut, gain of 8q) were associated with pure oligodendroglial diagnosis, but not with CFO. In the IDHwild tumors, some CNAs were associated with longer OS, however, there was no molecular factors associated with either pure oligodendroglial diagnosis or CFO. The pure oligodendroglioma histology might be associated with better OS in patients with IDHmut/Codel LrGGs but not in those with IDHmut/Noncodel or IDHwild LrGGs.

GENE-33. MECHANISM OF ACQUIRED MUTATION AFTER TMZ TREATMENT

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BACKGROUND: Glioblastoma (GBM) is poor-prognosis cancer and tumor recurrence is inevitable despite intensive chemoradiotherapy. Thus, insights into the mechanism of tumor recurrence are critical for improved patient treatment. Here, we describe our integrated genomic analyses using next-generation sequencing of the paired samples from initial and recurrent tumors. METHODS: We collected paired GBM samples in patients who recurred after temozolomide (TMZ) treatment. Mutation analysis of the cancer-related genes was performed using Ion Ampliseq Cancer Hotspot Panel v2. In addition, *MGMT* promoter methylation and expression of mismatch repair (MMR) protein such as MLH1, MSH2, MSH6, and PMS2 were analyzed by pyrosequencing and Western blotting, respectively. RESULTS: Sixty tumor samples from 29 patients were included in this study. Mutation acquisition of cancer-related genes was observed only in 12 (41%) patients while remaining 17 (59%) patients were mutationally stable even after TMZ treatment. Mutations were gained in 5 *MGMT* methylated tumors and 7 unmethylated. Remarkably, 70% of acquired mutations in *MGMT* methylated tumor were G: C>A: T at non-CpG sites whereas only 8% in *MGMT* unmethylated tumors. Acquired mutations in the recurrent sample after radiotherapy only were G: C>A: T at CpG site despite the tumor was *MGMT* methylated. In mutation gain group, MMR expression decreased significantly after treatment (p=0.0120.048). Furthermore, in contrast to *MGMT* unmethylated tumors, *MGMT* methylated tumors showed marked MMR inactivation (40% vs. 7.5%, p=0.078). Progression-free survival (PFS) and overall survival (OS) did not differ significantly between mutation-gain group and mutation-stable group (p=0.89 for PFS, p=0.67 for OS). CONCLUSIONS: We showed different types of the mutation acquisition after TMZ treatment according to *MGMT* status, providing further insights into the mechanism of TMZ resistance to improve treatment of the patients with GBM.

GENE-34. MOUSE MODEL OF DIFFUSE INTRINSIC PONTINE GLIOMA HARBORING Acvr1 G328V

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Diffuse intrinsic pontine glioma (DIPG) is pediatric brain tumor that occurs in the pons and for which there is no treatment. Mutations in Activin-A Receptor Type 1 (ACVR1), a bone morphogenetic protein (BMP) receptor, are highly recurrent and specific for DIPG. We used the Sleeping Beauty Transposase (SB) system to deliver plasmids encoding NRASV12 and a short hair pin for TP53 (shp53) with or without ACVR1-G328V, the most frequent of the six ACVR1 mutations found in DIPG, into the brainstem of neonatal mice, to generate endogenous tumors with DIPG mutations. Tumor development was monitored by measuring luciferase activity in vivo. Moribund stage animals were perfused and processed for histology. H&E staining demonstrated that the tumors developed in the brainstem and cerebellum. Tumors induced with NRAS/shp53/ACVR1 G328V had an increased median survival, MS = 129 dpi, compared to control group NRAS/

shp53, MS = 65 dpi (p=0.0002). In vitro and in vivo, tumors or tumor neurospheres (NS) harboring ACVR1-G328V exhibit elevated phospho-Smad1/5, transducer of the BMP pathway. RNA-seq analysis confirms that the TGF-Beta pathway is upregulated in tumor NS harboring ACVR1 G328V. From this data we also identified that signaling pathways involved in regulating pluripotency of stem cells and focal adhesion are differentially regulated. In summary, we used the SB transposase system to develop a mouse model that accurately represents the molecular biology of DIPGs with ACVR1 G328V mutations. Tumors arise in the brainstem and ACVR1 G328V prolongs survival through upregulation of the BMP-Smad1/5 pathways. In the future, we aim to elucidate the mechanisms by which ACVR1 contributes to tumor development to develop novel therapies for ACVR1 mutant DIPGs.

GENE-35. IDH1-R132H INDUCES AN EPIGENETIC REPROGRAMMING IN GLIOMA IMPACTING MEDIAN SURVIVAL, DNA-DAMAGE RESPONSE AND RADIO-SENSITIVITY

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IDH1-R132H is the most common mutation in lower-grade glioma and secondary glioblastoma. It is expressed in combination with ATRX and p53 inactivation in a mIDH1 glioma subtype which has been associated with better prognosis. We hypothesized that this is mediated by the impact of epigenetic changes on DNA-repair and DNA damage response (DDR) which maintain genome stability. To investigate this, we developed a mouse glioma model harboring IDH1-R132H, TP53-knockdown and ATRX-knockdown. Our results show that IDH1-R132H increases median survival (> 2 fold) compared with wt-IDH1 tumor bearing mice. Mutant-IDH1 tumors exhibit increased histone-3 methylation, and blocked neural cell differentiation. ChIP-seq, RNA-seq and Bru-seq data showed that genes involved in DNA-repair and DDR were upregulated in the mIDH1 mouse model. We also found differential enrichment of gene ontology terms related with DDR. Assessments of DNA-repair activity were performed in response to radiation (IR), i.e., H2AX and ATM phosphorylation, nuclear gH2AX and 53BP1 foci formation. These experiments were complemented with DNA-repair reporter assays. Our results demonstrate that mIDH1 mouse NS and human glioma cells exhibit increased DNA-repair and DDR activity, and enhanced genomic stability. This phenotype correlated with the response to radiation observed in vitro and in glioma bearing mice: IDH1-R132H expression in the genetic context of ATRX and TP53 inactivation elicits radio-resistance. Pharmacological inhibition of ATM or CHK1/2, two essential kinases in the DDR pathways, restored tumors' radio-sensitivity. This results were also validated in human glioma cells with endogenous expression of mIDH1, and in an alternative (NRAS independent) mIDH1 glioma model. In conclusion, genetically engineered mice harboring glioma encoding mIDH1, ATRX and TP53 knockdown displayed increased MS, increased DNA-repair, DDR and genomic stability, which hampers radiation efficacy. Our findings reveal a novel therapeutic approach for mIDH1 glioma patients, targeting DDR which would enhance the therapeutic efficacy of radiation.

GENE-36. ABERRANT ACTIVE-ENHANCERS ASSOCIATED WITH DOWNREGULATION OF HDAC1-RET FINGER PROTEIN COMPLEX OVERCOME CHEMORESISTANCE IN GLIOBLASTOMA

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RET finger protein (RFP) plays a pivotal role in the acquisition of chemoresistance via formation of a complex with nuclear transcription factor Y and histone deacetylase 1, producing specific enhancer activity. We hypothesized that chemoresistance mediated by RFP may result from aberrant deacetylation of H3K27 and dysregulation of novel *cis*-regulatory (active) enhancers. Therefore, we investigated the effects of RFP on active enhancers and gene expression and evaluated the effects of RFP depletion on the growth of glioma cells treated with temozolomide both *in vitro* and *in vivo*. We found that the combination of RFP depletion and TMZ treatment markedly suppressed the growth of glioma cells and extended the survival time of intracranial tumor-bearing mice compared to that of TMZ alone. Chromatin immunoprecipitation and whole-transcriptome sequencing revealed that RFP depletion weakened a significant number of enhancers, and diminished RNA with functions related to mitosis, DNA-dependent DNA replication and cell cycle. Further, the transcriptomes of FOXO1 and TBP2 were significantly increased, while that of PARP-binding protein (PARPBP) was decreased, resulting in induction of reactive oxygen species and cell death. This study suggests that RFP contributes to chemoresistance via aberrant deacetylation of histone H3K27 and dysregulation of RFP-associated active-enhancers in glioma and that the combination of targeting RFP and TMZ has potential as an effective novel treatment strategy for lethal glioma.

GENE-37. ATRX INACTIVATION DISRUPTS GLOBAL HETEROCHROMATIN LANDSCAPES AND ALTERS DISEASE-RELEVANT TRANSCRIPTIONAL ACTIVITY

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Diffusely infiltrating gliomas feature loss-of function mutations in the SWI/SNF chromatin remodeler gene *ATRX* as defining molecular alterations delineating major adult and pediatric disease subtypes. So far, *ATRX* inactivation in cancer has been mainly correlated with alternative lengthening of telomeres, however, we recently reported that Atrx deficiency drives glioma-relevant phenotypes, such as increased motility and astrocytic differentiation profiles, by directly modulating epigenomic landscapes and the corresponding transcriptional profiles in glioma cells of origin. In particular, Atrx deficiency was associated with disruptions in H3.3 histone content at key genetic loci. To further understand the downstream epigenomic dysfunction induced by ATRX deficiency, we compared genome-wide chromatin-state maps of Atrx+ and Atrx- primary murine neuroepithelial progenitors (mNPCs). This ChIP–seq analysis revealed major differences in the localization of heterochromatin repressive marks H3K9me3 and H3K27me3. Specifically, we identified peculiar locations in the genome displaying H3K9me3 depletion and gain of H3K27me3 upon Atrx inactivation. Interestingly, these regions were flanked by Atrx binding sites and perfectly co-localized with Lamina-Associated Domains (LADs). LADs are widely involved in the control of gene expression programs during lineage commitment and terminal differentiation; typically they have a cell-specific distribution and are very dynamic during differentiation stages. Gene Set Enrichment Analysis confirmed that the genes corresponding to newly formed LADs in mNPC-to-astrocyte differentiation are significantly enriched for genes down-regulated in Atrx deficient mNPCs and belonging to Gene Ontology categories such as cell cycle, chromosome organization and DNA damage. On the other hand, genes corresponding to decreased LAD association are enriched for up-regulated genes in Atrx- mNPCs and for regulation of differentiation, adhesion and cell death. These data are in perfect agreement with our previously described glioma-relevant phenotypes associated with Atrx deficiency and indicate a novel role of Atrx in regulating the spatial organization of heterochromatin and its underlying transcriptional activity.

GENE-38. INTRON 1-MEDIATED REGULATION OF EGFR EXPRESSION IN EGFR-DEPENDENT MALIGNANCIES

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The epidermal growth factor receptor (EGFR) gene is frequently altered in a variety of cancer types, with mutation and/or amplification occurring in approximately 57% of glioblastoma (GBM), 47% of non-small-cell lung cancer (NSCLC) and 31% of cancers of the head and neck (HNSCC). In these cancers EGFR protein overexpression is detectable in as much as 45%, 89% and 84% of these tumors respectively. This data suggests that transcriptional control of EGFR expression contributes to high EGFR protein levels in NSCLC and HNSCC, and may be a novel target to control EGFR expression in other dependent malignancies. In GBM, in which there is a stronger correlation between *EGFR* copy number and expression, targeting *EGFR* transcription may also be a viable approach for targeting tumors with high EGFR irrespective of copy number. Epigenetic mechanisms are critical for transcriptional regulation, however studies investigating the mechanisms regulating *EGFR* transcription are limited. Here we identify two novel super enhancers present in the first intron of the *EGFR* gene that we have termed *EGFR* super enhancers 1 and 2 (SE1, SE2). SE1 and SE2 span 37kb and 33kb respectively, contain H3K27Ac enhancer histone marks in NSCLC, GBM and HNSCC cells that functionally enhance transcription in reporter assays, and negatively impact *EGFR* transcript levels when perturbed by CRISPR/Cas9 guided deletion. Using locally-generated ATAC-seq and transcription factor ChIP-seq from the ENCODE consortium we have identified putative binding sites for AP-1 transcription factor subunits in critical constituent enhancers within SE1 and SE2. Disrupting the binding of these transcription factors through site directed mutagenesis negatively impacts the enhancer function in reporter assays. Our results identify and characterize these novel enhancers, shedding light on the role that epigenetic mechanisms

play in regulating *EGFR* transcription in EGFR-dependent cancer types and presents a novel angle by which these malignancies can be treated.

GENE-39. MicroRNA-BASED MARKERS IN CEREBROSPINAL FLUID AS POTENTIAL DIAGNOSTIC TOOLS FOR LEPTOMENINGEAL METASTASIS IN LUNG ADENOCARCINOMA PATIENTS

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BACKGROUND: We hope to identify leptomeningeal metastasis (LM)-related cerebrospinal fluid (CSF) miroRNAs as potential molecular markers contributing to diagnosis of LM patients with lung adenocarcinoma. MATERIAL AND METHODS: CSF samples were collected from lung adenocarcinoma patients with LM (positive group) and non-LM patients (negative group). Meanwhile, matching CSF samples from other lung adenocarcinoma LM patients were collected before and after effective LM-related treatment. Samples were screening using Agilent Human microRNA arrays. Differentially expressed microRNAs in both of two sets as well as significantly expressed microRNAs in either one set were selected as targets. Whereafter, expression of target CSF miRNAs was determined using quantitative real-time PCR (qRT-PCR). Differential microRNAs expression was analyzed by Mann-Whitney Test. Receiver Operating Characteristic (ROC) curve analysis was used to determine sensitivity and specificity. RESULTS: Twenty samples obtained from 10 LM patients and 10 non-LM controls (5 brain metastasis from lung adenocarcinoma and 5 nonneoplastic disease) were subjected to microRNA arrays. Meanwhile, 6 couples of matching samples were subjected to microRNA arrays.The intersection of significantly differential expression, miR-7977, miR-7975 and miR-4800-5p, were significantly up-regulated in positive group, and down-regulated after effective LM-related treatment. These three microRNAs were selected as targets and subjected to qRT-PCR. Significant increase was noticed in expression of miR-7977 (P=0.001), miR-7975 (P=0.001) and miR-4800-5p (P=0.024) in newly collected samples of 17 LM cases compared with 14 non-LM controls (9 brain metastasis from lung adenocarcinoma and 5 benign brain tumor). ROC analysis indicated that area under curve for miR-7977, miR-7975 and miR-4800-5p was 0.849(P=0.001, 95%CI 0.694–1.0), 0.840(P=0.001, 95%CI 0.688–0.992), and 0.739(P=0.024, 95%CI 0.564–0.915), respectively. The cutoff value, sensitivity and specificity were 48.3, 0.824 and 0.857 for miR-7977, 29.4, 0.824 and 0.929 for miR-7975, and 44.9, 0.765 and 0.643 for miR-4800-5p, respectively. CONCLUSION: MiR-7977, miR-7975 and miR-4800-5p may serve as molecular markers for diagnosis of LM from lung adenocarcinoma.

GENE-40. MEGF10, A GLIOMA SURVIVAL ASSOCIATED MOLECULAR SIGNATURE PREDICTS IDH MUTATION STATUS

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BACKGROUND: Glioma is the most common primary brain tumor with various genetic alterations, among which IDH mutation is the most common mutation and plays an important role in glioma early development, especially in lower grade glioma (WHO II-III). Previous studies have found that IDH mutation is tightly associated with extensive methylation across whole genome in glioma. METHODS: To investigate the role of IDH, we obtained methylation data of 777 samples from CGGA (Chinese Glioma Genome Atlas) and TCGA (The Cancer Genome Atlas) with IDH mutation status available. A package compiled under R language called *Tspair*, was used as the main analytic tool to find potential probes that were significantly affected by IDH mutation. ROC analysis, Kaplan-Meier analysis, Gene ontology analysis and Gene Set Variation Analysis were further performed to explore the clinical values and biological functions of the candidate gene. RESULTS: We found one pair of probes, cg06940792 and cg26025891 was capable of predicting IDH mutation status precisely. The hyper-methylated probe was cg06940792, designed in the promoter region of MEGF10 while the hypo-methylated probe was cg26025891, designed in the promoter region of PSTPIP1. Survival analysis proved that hypermethylation or low expression of MEGF10 indicated a favorable prognosis in 983 glioma samples. Moreover, gene ontology analysis demonstrated that MEGF10 was associated with cell migration, cell proliferation and regulation of apoptosis in glioma. All findings above can be validated in three other independent cohorts. CONCLUSIONS: In a word, our results suggested that methylation level and mRNA expression of MEGF10 in glioma was not only correlated with IDH mutation, but also associated with clinical outcome of patients, providing potential guide for future dissection of IDH role in glioma.

GENE-41. LIQUID BIOPSY USING CELL FREE DNA FROM THE CEREBROSPINAL FLUID (CSF) IN GLIOMA

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Mutation in the *IDH1*gene is a genetic defect exclusively found in glioma, and has been established as a prognostic factor for WHO Grade 2–4 glioma. Therefore, detecting this mutation is clinically important. In this study, we report the results of analyzing defects in *IDH1* mutation by detecting circulating cell free (ccf) DNA in the CSF derived from the tumor tissue of glioma patients. Moreover, *MGMT*promoter methylation, *H3F3A* and *BRAF*mutation were evaluated for the CSF samples in a subset of glioma patients. Lumbar puncture was performed to obtain CSF from 7 patients with glioma. ccfDNA was extracted from 1 ml of CSF using the Maxwell rapid sample concentrator system. Subsequently, the presence of point mutation of the*IDH1*gene was screened by real-time PCR/high-resolution melting (HRM) curve analysis, and the mutation was confirmed on the basis of DNA sequencing results. Status in the *IDH1*gene was also analyzed using the same assay technique for the DNA extracted from the excised fresh tumor tissue, to compare the results with that of CSF-derived ccfDNA. In addition, ccfDNA was also extracted from the plasma in 3 patients to additionally examine the presence of the *IDH-1*gene mutation. *MGMT*promoter methylation, *H3F3A* and *BRAF*mutation were also performed using the HRM method. CSF-derived ccfDNA was successfully extracted from all patients and analyzed. *IDH1*gene mutation was detected in 3 of the 7 glioma patients. The results of the *IDH1*gene analysis of CSF-derived ccfDNA and that of the DNA extracted from the surgically excised tumor tissue were consisted in all patients. Three *MGMT*promoter methylation, two *H3F3A*mutations, and three *BRAF*mutations were detected, respectively. Gene analysis of ccfDNA from the CSF enabled the evaluation of *IDH1* mutation in glioma patients less invasively, without directly obtaining any tumor tissue. Moreover, this technique can be applied to analyze *MGMT*-gene promotor methylation, *H3F3A* and *BRAF*mutation.

GENE-42. THE GENOMIC LANDSCAPE OF TRIPLE-NEGATIVE GLIOBLASTOMA

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Glioblastoma (GBM) is the most common and deadly primary malignant brain tumor in adults. Mutations in the *TERT* promoter (*TERTp)* and isocitrate dehydrogenase 1 or 2 (*IDH1/2*) can classify ~80% of GBMs into molecular subgroups with distinct clinical courses. These molecular subgroups utilize distinct genetic mechanisms of telomere maintenance, either *TERTp* mutation leading to telomerase activation or *ATRX*-mutation leading to an alternative lengthening of telomeres phenotype (ALT). However, approximately 20% of GBMs lack alterations in *TERTp* and *IDH1/2*. These tumors, designated *TERTp*^{WT}-*IDH*^{WT} or triple-negative glioblastomas (as they also lack 1p19q co-deletion) do not have well-established genetic biomarkers or defined mechanisms of telomere maintenance. Here we performed whole-exome, whole-genome, and RNA-sequencing on a cohort of triple-negative GBMs to define their genetic landscape. We discovered recurrent inactivating mutations in SWI/SNF-related, matrix-associated, actin-dependent regulator of chromatin, subfamily A-like 1 (*SMARCAL1*) in 21% (8/39) of triple-negative GBMs. Using telomere maintenance characterization assays, we show that *SMARCAL1*-mutant cases exhibited the ALT phenotype. Using CRISPR/Cas9 gene editing in GBM cell lines, we found that inducing loss of SMARCAL1 generates features of ALT. Rescue of expression of SMARCAL1 in *SMARCAL1*-null cell lines markedly suppressed ALT features and was dependent on the enzyme helicase activity. Furthermore, using break-apart FISH and whole genome sequencing, we identified recurrent rearrangements upstream of *TERT* in ~50% of triple-negative GBMs. These *TERT*-rearranged tumors exhibited elevated levels of *TERT* mRNA expression. This represents a novel mechanism of telomerase activation in GBMs lacking the well-known *TERT* promoter hotspot mutations. Finally, we identify recurrent BRAF V600E mutations in younger patients with GBM. Collectively, our findings define novel molecular subgroups of glioblastoma, including a telomerase-positive subgroup driven by *TERT*-structural rearrangements (*IDH*^{WT}-*TERT*^{SV}, ~50%), and an ALT-positive subgroup (*IDH*^{WT}-ALT, ~40%) with mutations in *ATRX* or *SMARCAL1*. We also establish *SMARCAL1* inactivating

HEALTH OUTCOME MEASURES

HOUT-01. A RETROSPECTIVE ANALYSIS OF OUTCOMES WITH TEMOZOLOMIDE AS INITIAL TREATMENT OF GRADE 2 OLIGODENDROGLIOMA

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INTRODUCTION: Prior randomized studies have shown a survival benefit of radiation and chemotherapy compared to radiation alone for grade 2 gliomas (astrocytoma and oligodendroglioma) and grade 3 oligodendroglioma. However, data is mixed regarding outcomes for patients treated initially with chemotherapy alone as well as outcomes based on sequence of chemotherapy and radiation treatments. METHODS: We performed a retrospective analysis of outcomes in grade 2 oligodendroglioma treated initially with temozolomide (TMZ) alone. Variables included in the analysis were age, extent of resection, timing of TMZ treatment, progression free survival, and overall survival. RESULTS: A total of 37 patients with grade 2 oligodendroglioma who received TMZ as the first course of treatment were identified. Median age at diagnosis was 43 years. Extent of resection was as follows: complete resection 32%, subtotal resection 43%, stereotactic biopsy 14%, and unknown in 11%. Of the 37 patients in the cohort, 41% received TMZ immediately following initial diagnosis with the remaining 59% receiving TMZ after initial observation. Median PFS after TMZ treatment was 3.94 years, and 25% of patients treated with TMZ alone progressed within 2 years. Despite the high rate of early progressors, the median overall survival with TMZ alone as initial treatment was 15.46 years Extent of resection was not significantly associated with survival. CONCLUSIONS: This retrospective series supports the observations from the TMZ monotherapy exploratory arm of CODEL that PFS in oligodendroglioma patients treated with TMZ alone as initial therapy is likely significantly worse than patients treated with combination of radiation and chemotherapy. The relatively good OS in this same patient population suggests that additional salvage therapy at the time of progression may still result in prolonged survival. Clinical and molecular biomarkers are needed to identify higher or lower risk subtypes within oligodendrogliomas to assist in treatment decision making.

HOUT-02. VARIATION IN POST-OPERATIVE LENGTH OF STAY IN NEURO-ONCOLOGIC SURGERY

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INTRODUCTION: Little is known about the predictors of post-operative length of stay (LOS) following brain tumor resection or the influence of LOS on post-operative outcomes. METHODS: We assessed associations between LOS and 30-day post-operative morbidity, mortality, and readmission in a nationwide retrospective cohort study of Americans who underwent resection of an intracranial tumor. The analysis included data collected from the NSQIP 2006–2015 and NHDS 1970–2010 registries in a multivariable logistic regression. RESULTS: 16,101 craniotomy for tumor cases were identified in NSQIP with a median age of 58 years (IQR 47–67). Median post-operative length of stay was 3 days (IQR 2–6). Patients with LOS of 1–2 days after craniotomy were deemed early discharge (26%), 3–5 days was considered an intermediate LOS (46%), while 6+ days of LOS was considered late discharge (28%). Predictors of early discharge included male sex, white race, young age, functional independence, low chronic disease burden, low ASA score, shorter operative length, supratentorial location, and cranial nerve tumor histology (p CONCLUSION: Early discharge following craniotomy for tumor resection was associated with decreased likelihood of post-operative medical complications, readmission, and death as compared to late discharge. Select patients may benefit from personalized discharge planning that prioritizes shorter LOS.

HOUT-03. SCREENING FOR MOOD DISTURBANCE IN LONG-TERM CENTRAL NERVOUS SYSTEM (CNS) TUMOR SURVIVORS USING PATIENT REPORTED OUTCOMES MEASUREMENT INFORMATION SYSTEM (PROMIS): A NEURO-ONCOLOGY BRANCH NATURAL HISTORY STUDY (NOB-NHS) REPORT

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BACKGROUND: Survivorship is an important area of cancer care and research. There are limited studies exploring the experience of long term survivors of CNS tumors. A recent review of quality of life in adults with CNS tumors underscored the need for studies exploring patient outcomes, including mood disturbance. This report explores mood disturbance in CNS tumor patients living greater than 5 years from diagnosis. METHODS: Patients enrolled on the NOB-NHS were included. PROMIS depression/anxiety measures were completed at study entry. T-scores >60 were considered significant. Independent sample t-tests, chi-square and Fishers Exact tests were used to identify associations with mood disturbance. Significance level was set at 0.05. RESULTS: 132 patients, primarily white (84%), males (58%), with median age 49 (22–81), were included with 59% having low grade tumors; oligodendroglioma (19%) was the most common diagnosis, 30% underwent gross total resection, 33% had had a recurrence and 27% had a poor KPS (80). Five percent were on corticosteroids and 20% on psychotropic medications. Overall, 14% and 18% reported significant depression and anxiety respectively, with 10% reporting both. More non-white [29% vs 11%; X2 (1) = 4.4, p<0.04] reported significant depressive symptoms, as did those with poor KPS [25% vs. 10%; X2 (1) = 4.2, p<0.04] and those on psychotropic medications [27% vs 11%; X2 (1) = 4.5, p<0.04]. Anxiety was only associated with the use of psychotropic medications [38% vs. 13%; X2 (1) = 9.3, p<0.01]. CONCLUSION: Symptoms of depression and anxiety occurred in 20% of long-term brain tumor survivors on screening. Depressive symptoms were associated with race, lower KPS, and use of psychotropic medications. Additionally, use of psychotropic medications was associated with anxiety. Assessment of depression and anxiety as part of survivorship care is warranted. Future studies exploring phenotypes at risk and targeted interventions are needed to mitigate these symptoms.

HOUT-04. DEMOGRAPHIC PROFILES, MANAGEMENT AND CLINICAL OUTCOMES OF GLIOBLASTOMA PATIENTS TREATED AT ST. LUKE'S MEDICAL CENTER- PHILIPPINES

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INTRODUCTION: Glioblastoma is a highly aggressive primary brain tumor and typically treated with maximal safe resection followed by concomitant radiation and temozolomide followed by 6 cycles of adjuvant temozolomide. METHODS: We reviewed histologically documented glioblastoma cases seen and treated at our center from 2005 until 2017. Demographic data, treatments received, PFS and OS were collected. RESULTS: 105 GBM patients were treated with a median age of 45 and KPS 90. Fifty five percent (58/105) had gross total resection, 18% subtotal resection and 13% biopsy. Seventy percent (74/105) were treated with RT plus TMZ, 15 %(16/105) RT alone and 1 patient treated with TMZ alone. The most common adverse events are fatigue and somnolence. Median follow up is 10.5 months. Progression free survival (PFS) is 13.38 months while overall survival (OS) is 15.43 months. CONCLUSION: This is the first study done for glioblastoma patients in the Philippines treated after surgery with RT plus concomitant and adjuvant TMZ. The results are consistent with the published data.

HOUT-05. INVESTIGATION OF Ki-67 PROLIFERATIVE INDEX AND PATIENT SURVIVAL IN GLIOBLASTOMA MULTIFORME

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INTRODUCTION: Glioblastoma multiforme (GBM) is a rapidly growing, aggressive brain tumor. Studies demonstrate that higher Ki-67 proliferative indices predict poorer survival in GBM patients. It remains unclear whether therapeutic responses vary when stratifying patients by Ki-67 index. Our objective was to investigate the relationship between Ki-67 index and post-surgical survival in patients with GBM undergoing radiotherapy and/or chemotherapy. METHODS: Patients with GBM presenting to VCU Health between January 2005-February 2015 were retrospectively reviewed. Inclusion criteria were: 1) Age > 18, 2) Biopsy/surgery with histopathological testing, 3) Reported Ki-67 index, and 4) Initial T2 post-contrast tumor volumes. Ki-67 indices were stratified into three groups: Ki-67 ≤ 10, 10 < Ki-67 ≤ 20, and Ki-67 > 20. The primary outcome was overall survival, calculated

as time between surgery and death. Cox proportional hazards regression compared the relationship between Ki-67 index and overall survival after controlling for age, sex, race, marital status, medical comorbidities, initial T2 tumor volume, radiotherapy and chemotherapy. RESULTS: There were 69 patients in the study. Demographics, comorbidities, radiotherapy and chemotherapy did not differ between Ki-67 groups. Median survival time (weeks) was 61.7 (32.9–132.6), 34.9 (13.6–84.9), and 39.3 (16.7–84.1) for Ki-67 ≤ 10, 10 < Ki-67 ≤ 20, and Ki-67 > 20 groups, respectively. There was no significant difference in survival between Ki-67 groups in unadjusted (p=0.69) or adjusted analyses (p=0.83). However, older age (1.06 hazard ratio, 1.01–1.11 CI, 0.01 p-value), radiotherapy (91.5, 4.38-1911.27, 0.004), arthritis (7.81, 1.30–46.79, 0.03), diabetes (7.22, 42.14, 0.03), and hypercholesterolemia (15.76, 3.40–72.97, < 0.001) were associated with shorter survival. CONCLUSIONS: This study demonstrated no relationship between Ki-67 index and survival in patients with GBM when controlling for other factors, but highlighted factors related to poorer survival (older age, radiotherapy, arthritis, diabetes, hypercholesterolemia). Further prospective studies on the association between survival and Ki-67 index in a larger cohort of patients with GBM are warranted.

HOUT-06. PATTERN OF LOW FIELD INTENSITY RECURRENCE IN HIGH-GRADE GLIOMAS FOLLOWING TUMOR TREATMENT FIELD THERAPY

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BACKGROUND: Glioblastoma (GBM) is an aggressive neoplasm that continues to show recurrence despite recent advances in therapy. Tumor Treating Fields (TTFields) is a non-invasive, regional antimitotic treatment modality, delivering low-intensity (1–3 V/cm), intermediate-frequency (100–300 kHz), alternating electric fields to tumors via transducer arrays placed on the scalp of GBM patients. TTFields disrupts cell division processes in cancer cells. However, some mechanisms of escape have been described, in particular, out-of-field tumor recurrence. The brainstem dose not receive high intensity electric field stimulation during TTFields therapy, and represents one possible region of tumor recurrence following treatment. METHODS: Six cases were identified via retrospective chart review from multiple providers that have experience with prescribing and planning treatment with TTFields. Clinical and radiographic reviews were performed after de-identification of patient records. Cases were compared according to demographics, tumor location and region of recurrence. RESULTS: The median patient age at diagnosis was 62 years (Range: 10–69) with 3/6 patients being male and 3/6 patients being female. Tumors were initially located in the frontal (3/6), parietal (2/6), and temporal lobes (1/6). Three patients received TTFields therapy at initial diagnosis in combination with surgery and chemoradiation, while the remaining patients received TTFields therapy at initial recurrence in conjunction with bevacizumab. Following TTFields treatment, tumors recurred predominantly in the pons (1/6), left cerebellar peduncles (3/6), and right cerebellar peduncles (2/6). The average time to brainstem recurrence following TTFields therapy was 152 days (Range: 49–228). CONCLUSIONS: Treatment options for patients with GBM are limited, however TTFields has been shown to improve overall survival when combined with temozolomide. TTFields can only be delivered in therapeutic intensities to the supratentorial brain. Brainstem recurrence may occur as field intensity in the brainstem is sub-therapeutic. Further investigation is warranted to determine optimal treatment for brainstem recurrences for patients receiving TTFields therapy.

HOUT-07. AN EVALUATION OF THE TREATMENT COURSE IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

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INTRODUCTION: Glioblastoma multiforme (GBM) is a malignant brain tumor with a mean overall survival of 15 months using current treatment modalities. Previous studies have shown delays in treatment result in worse outcomes. Demographic and socioeconomic factors may have a role in treatment course variations. Therefore, the objective of this study was to investigate factors that influence time to treatment and effect overall survival in patients with GBM. METHODS: Records from patients who were diagnosed with GBM and underwent surgery, radiotherapy, or chemotherapy at VCU Health between 2005 and 2015 were retrospectively reviewed. Linear regression was used to determine whether demographics, socioeconomic status, or presenting symptoms were associated with the time from first clinic visit to time of surgery, chemotherapy, radiotherapy first treatment, and time until all treatments were initiated. Then, cox proportional hazards regression assessed whether these five outcomes were independently related to overall survival. RESULTS: 126 patients were included in the study. Median survival was 10.6 months. None of the demographic factors or presenting symptoms were associated with time to treatment outcomes. Time to radiotherapy, chemotherapy, and all treatment initiation were significantly associated with overall survival after controlling for age, tumor location, and extent of resection (p< 0.01). A thirty-day decrease in time to radiotherapy, chemotherapy, and all treatment initiation were associated with increased mortality hazards of 1.05, 1.08, and 1.04, respectively. DISCUSSION: Our results indicated that demographics, socioeconomic factors, and presenting symptoms do not contribute to delays in treatment. Our study also revealed that, contrary to current literature, patients with shorter time to initiation of treatment had poorer outcomes. This could represent a specific population that presents later in their disease course and thus receives more aggressive treatment. Further investigation is therefore needed to elucidate the cause of this relationship and factors causing decreased survival in this population.

HOUT-08. COMORBID MEDICAL CONDITIONS AS PREDICTORS OF OVERALL SURVIVAL IN SURGICAL PATIENTS WITH GLIOBLASTOMA

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INTRODUCTION: Glioblastoma multiforme (GBM) is a common, fast-growing central nervous system tumor with poor prognosis. Survival from diagnosis to death is estimated to range from 7.2 to 26.6 months. Only a few predictors of survival have been well-established, including age, extent of resection (EOR), performance status, and MGMT status. Our objective was to determine whether demographic characteristics and comorbid medical conditions influence overall survival (OS) in surgical GBM patients. METHODS: Data came from the Virginia Commonwealth University (VCU) Brain and Spine Tumor Data Registry, which collects retrospective information on all patients who presented to VCU Health with a CNS tumor between January 2005 and February 2015. Patients diagnosed with GBM who underwent surgery and/or biopsy were included in this study. Cox proportional hazards regression controlling for age, EOR, and tumor location was used to assess the relationship between OS and sex, race, insurance status, marital status, alcohol/tobacco use, initial tumor volume, and comorbidities. Individual analyses were performed for each predictor and those with p<0.15 were entered into multivariate models. RESULTS: There were 163 patients who met inclusion criteria. Mean OS was 10.6 months with a survival probability of 66% at 6 months, 46% at 12 months, and 21% at 24 months. After individual analysis, sex (HR: 1.37, 95% CI: 0.98–1.92, p=0.07) and history of asthma (HR: 2.04, CI: 0.98–4.28, p=0.06), hypercholesterolemia (HR: 1.69, CI: 0.95–3.01, p=0.07), and depression/anxiety (HR: 1.81, CI: 1.04–3.16, p=0.04) were included in the final model. In multivariate modeling, no demographic characteristics or comorbidities were significantly associated with OS. CONCLUSIONS: History of depression/anxiety was significantly associated with OS on individual analysis. However, this association did not remain significant in final multivariate modeling. The potential link between depression/anxiety and survival in GBM patients needs further evaluation in studies with greater power, given this could be amenable to intervention.

HOUT-09. USING THE ASCO AND ESMO FRAMEWORKS TO ASSESS THE CLINICAL VALUE OF TUMOR TREATING FIELDS FOR NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME

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BACKGROUND: The effectivity and safety of TTFields in newly diagnosed GBM was recently demonstrated by the final analysis of the large, randomized controlled EF-14 Trial. To capture the clinical value of new cancer treatments, the American Society of Clinical Oncology (ASCO) and the European Society for Medical Oncology (ESMO) have both developed assessment frameworks. We quantified the clinical value of the TTFields treatment in GBM by applying ASCO and ESMO frameworks to the EF-14 trial data. MATERIALS/METHODS: The EF-14 Trial (n=695) proved the effect of adding TTFields to maintenance temozolomide (TMZ) for newly diagnosed glioblastoma patients. The ESMO Magnitude of Clinical Benefit Scale (MCBS) and the ASCO Net Health Benefit (NHB frameworks provide separate calculations for adjuvant therapies and treatments for advanced diseases. We applied both classifications to the EF-14 trial data as required by the framework rules. Quality of life data from the EF-14

Trial was also included. Additionally, we identified reference points from the literature to understand the NHB and MCBS results for TTFields in GBM. RESULTS: Applying the ASCO framework to the EF-14 data resulted in a NHB score of 56/62 (adjuvant/advanced). Those were among the highest scores identified in the literature search. Additionally, the ASCO framework valued the reduction in cancer-related symptoms (e.g., pain, weakness of legs) with TTFields. Applying the ESMO framework resulted in MCBS scores of A/5 (adjuvant/advanced), which are the highest scores achievable in the ESMO framework. The ESMO framework valued the Health Related Quality of Life (HRQoL) gain during deterioration-free survival time with TTFields. CONCLUSIONS: Both the ASCO and ESMO frameworks suggest, that adding TTFields to maintenance TMZ for newly diagnosed glioblastoma patients provides patients with significant clinical benefits. The high scores underline the fact, that treatment with TTFields extended progression free and overall survival without additional systemic toxicities.

HOUT-10. SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) TREATMENT IS ASSOCIATED WITH IMPROVED SURVIVAL AMONG ELDERLY PATIENTS DIAGNOSED WITH GLIOBLASTOMA

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INTRODUCTION: Glioblastoma (GBM) is an incurable primary brain tumor with a median age of diagnosis at 64 years old. Previous studies have suggested an association between psychosocial factors and GBM patient outcomes. Here, we retrospectively investigated the relationship between SSRI treatment and overall survival (OS) in deceased GBM patients previously cared for in our hospital system. METHODS: Medical records of 449 patients diagnosed with GBM after the year 2000 were obtained from our hospital systems data warehouse. Patient ages ranged from 18 to 86 (median=59 years old) and were 60.6% male. All patients received temozolomide and were excluded if they had a clinical history of progression from a lower-grade glioma. Patients were stratified into 2 groups: (i) with or (ii) without SSRI treatment (defined as at least 1 recorded SSRI prescription post-diagnosis). OS was analyzed with a Kaplan-Meier survival curve and compared with the log-rank test. RESULTS: GBM patients with SSRI treatment (n=151) have an increased median OS of 537 days, as compared to 431 days among untreated patients (n=298) (P=0.0011). Stratifying by age reveals a striking OS benefit, with a median survival of 486 days among elderly (60 years old) patients treated with an SSRI (n=83) versus untreated patients (n=137) with a median survival of 329 days (P=0.0009). Although less robust, there is also a 59.5 day median OS improvement in younger (<60 years old) GBM patients treated with SSRIs (n=68), as compared to untreated individuals (n=161) (P=0.0379). CONCLUSIONS: GBM patients treated with SSRIs possess significantly improved OS, with an enriched effect among elderly individuals for whom therapies are often less effective and/or limited. These preliminary results require further modeling to definitively interpret the direct effects of SSRIs on GBM and the immune response against GBM, while we also plan clinical trials to further elucidate these exciting observations.

HOUT-11. ECONOMIC IMPACT OF SODIUM THIOSULFATE ON CISPLATIN-INDUCED HEARING LOSS

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BACKGROUND. Cisplatin-induced hearing loss (CIHL) develops in majority of children receiving cisplatin chemotherapy. Recently two phase-3 clinical trials demonstrated effectiveness of sodium thiosulfate (STS) in preventing CIHL. This study quantifies the potential economic impact of STS on cisplatin-treated children with hepatoblastoma (HB) in US. METHODS. We used an incidence-based approach to model CIHL cost for standard risk HB patients 19 yrs. Input data included: 1) estimates of incidence and survival, and age distribution of HB in US; 2) incidence of CIHL and effectiveness of STS; 3) lifelong health care and education cost and lost productivity (up to 65 yrs). Market cost of STS is unknown and not included but expected to be a small part of lifelong cost. When estimating the total and reduced costs, costs of CIHL were calculated by age and degree of CIHL severity (none, mild, severe), and the uncertainty in the input estimates were incorporated through Monte Carlo simulation. RESULTS. For a child diagnosed with HB at 1yr old and developed severe CIHL, the estimated lifelong cost is $825K ($347K health care and education cost; $478K lost productivity). Given an incidence of 0.13 cases per 100,000 in US, the annual cost of CIHL is estimated to be $16.7 million (95% CI 15.5–17.8). Assuming that all new HB patients receive STS and CIHL is reduced from 70% to 30%, the annual reduced cost in the US would be $1.81million (95% CI 1.29–2.34) after 5 years, $3.60million (95% CI 2.87–4.37) and $6.96million (95% CI 5.98–8.03), respectively, after 10 and 20 years. CONCLUSION. STS treatment could potentially lead to significant reduction in CIHL-related cost to society. Further analyses will evaluate the economic impact of STS on all childhood solid tumors, and include STS cost. Such economic impact will help inform the future clinical role of STS.

HOUT-12. CHARACTERIZATION OF SYMPTOM BURDEN IN MINORITY PATIENTS WITH CNS TUMORS: A REPORT FROM THE NEURO-ONCOLOGY BRANCH (NOB) NATURAL HISTORY STUDY (NHS)

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Primary CNS tumor patients are highly symptomatic, with 40% of brain tumor patients reporting 3 moderate-severe symptoms. The five most commonly reported are fatigue, drowsiness, difficulty remembering, disturbed sleep in brain and in spine patients, pain, numbness, leg weakness, and autonomic dysfunction (bowel and sexual dysfunction). Twenty-five percent reported interference with activity and mood. These reports are limited to primarily white patients, with limited studies exploring in more diverse samples. Fifty patients self-identified as non-white are included in this report of symptom burden (MD Anderson Symptom Inventory-Brain and Spine Tumor), depression and anxiety (PROMIS measures). Descriptive statistics, and standardized classification of severe symptoms and mood are used to describe the sample characteristics Chi-square and Fishers exact test were used to identify associations. Significance level was set at $p < 0.05$. The majority of patients were male(62%), Black/African American(38%). Median age 40(range 14–69), 44% had a gross total resection at diagnosis. Glioblastoma was the most common diagnosis(40%) with 1/3 having a poor(KPS 80) and a recurrence, and 56% having completed treatment. All spine and 34% of brain tumor patients had at least three symptoms rated as moderate-severe. The top five among brain tumor patients were weakness in arms/legs, fatigue, disturbed sleep, difficulty remembering, and feeling irritable and 57% reported moderate-severe activity related interference from symptoms. Fourteen percent of patients reported having moderate-severe depression and anxiety symptoms at study entry. Diagnosis other than glioblastoma was associated with depression symptoms ($X2(1) = 5.4$, $p < 0.04$), but no associations between clinical and demographic factors were found with interference, depression, or anxiety. Minority populations are highly symptomatic, but differences in symptoms reported as most severe were found when compared to historical data which is a restricted patient demographic. Future studies exploring symptom prevalence, underlying risk and treatment in diverse populations are warranted.

HOUT-13. POSTOPERATIVE HEARING PRESERVATION IN PATIENTS UNDERGOING RETROSIGMOID CRANIOTOMY FOR RESECTION OF VESTIBULAR SCHWANNOMAS: A META-ANALYSIS OF 1,249 PATIENTS

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BACKGROUND: Vestibular schwannomas (VS) are slow-growing, benign tumors originating from over-proliferation of Schwann cells ensheathing the vestibulocochlear nerve. Sensorineuronal hearing loss is the most common presenting symptom. RESEARCH QUESTION: To investigate estimated hearing preservation (HP) rates in VS patients after retrosigmoid (RS) surgery through a meta-analysis based on published hearing outcomes. METHODS: PubMed and Cochrane were used to identify applicable retrospective and prospective studies. Study heterogeneity was quantified through t2, Q, and I2 statistics. A Wald-type Q statistic was used to assess statistical significance of study heterogeneity. Assessment of study bias was performed using standard funnel plot analysis and an Eggers test for funnel plot asymmetry. RESULTS: Significant cross-study heterogeneity was found, with rates of HP ranging from 12–79% across studies. Aggregate HP was 23% under a fixed effects model and 37% under random study effects. Clear systematic bias was also apparent, with disproportionate numbers of studies reporting HP rates markedly higher than the aggregate estimates ($P < 0.0001$). As expected, rates of HP were also strongly dependent

on preoperative tumor size, with rates of 59%, 37%, and 11% observed for intracanalicular, small, and large (>20 mm) tumors, respectively. SIGNIFICANCE: HP rates are likely dependent on multiple factors, with tumor size as a strong effect. It is critical to discuss the patients expectations for HP when deciding treatment plans for VS.

HOUT-14. HOMOLOGOUS RECOMBINATION DEFICIENCY IN PATIENTS WITH HIGH GRADE GLIOMAS

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Patients with Homologous Recombination Deficits (HRD) have shown increased survival in many cancers. With PARP inhibitors surfacing as a potential therapy for Glioma patients, we aim to explore the existence of HRD and its prognostic value in High-Grade Glioma patients. We conducted a chart review of patients with Gliomas who received Foundation One testing at The University of Oklahoma from 2013 to 2018. We used Foundation Ones Loss of Heterozygosity (LOH) score as a measure for HRD. We analyzed the correlation between the existence of mutated IDH and the LOH score. We then analyzed the significance of receiving Foundation One testing prior to any treatment on LOH score. Finally, we separated the patient population into two groups, High-HRD (>5% LOH or >3% LOH) and Low-HRD (<5% LOH or <3% LOH), and used the Kaplan-Meier method to assess OS and PFS. 39 patients were included in the final analysis. The relationship between IDH status and LOH score approached significance (P=.09). The relationship between time of foundation testing and LOH score was statistically significant (P=.05). In analyzing survival, we were only able to find significance in patients who received genetic testing post-treatment. Interestingly, the average OS of the Low-HRD group was 19 months, compared to 52 months for the High-HRD population which was statistically significant (p=.02). There appears to be a relationship between IDH status and HRD and although it was not yet statistically significant, it approached significance. Patients tested in a recurrent setting were more likely to possess higher HRD. We found no statistically significant difference in PFS or OS between the low-HRD and high-HRD populations, except in the later stage patient population. Due to the difference in the average PFS and OS this should be studied further in a larger population.

HOUT-15. CIRCULATING BLOOD CELL COUNTS AS POTENTIAL BIOMARKERS OF OUTCOMES IN RECURRENT GLIOBLASTOMA PATIENTS TREATED WITH BEVACIZUMAB

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OBJECTIVE: There is a lack of biomarkers to identify patients who may benefit from specific salvage therapies in glioblastoma (GBM), such as from the anti-angiogenic agent bevacizumab. We have recently shown that leukopenia is a potential biomarker for overall survival (OS) in patients with newly diagnosed glioblastoma treated with chemoradiation. We hypothesize that circulating blood counts may also serve as a potential biomarker of overall outcome in patients with progressive GBM treated with bevacizumab. METHODS: Complete blood counts (WBC, RBC, and PLT, and relative blood counts of monocytes, neutrophils, eosinophiles and basophiles) and clinical and radiographic information were collected retrospectively from GBM patients with progressive disease and treated with bevacizumab monotherapy. RESULTS: 92 patients (60.87% male and 39.13% female) with progressive GBM were identified. The length of bevacizumab (10mg/kg q2 weeks) ranged from 21 to 984 days (M = 152.48d SD = 158.67d). OS following initiation of bevacizumab ranged from 41 to 984 days (M = 233.94d SD = 176.42d). Using a cox proportional hazard model, we found no significant changes in total white blood counts, red blood counts, lymphocytes, neutrophils, basophils, eosinophiles and monocytes over the course of bevacizumab therapy. Unlike our prior findings in newly diagnosed GBM, WBCs did not show any correlation with overall outcome (OS, PFS). However, we found that increases in platelet counts during bevacizumab therapy were associated with a strong trend in improved OS (p=0.059). Age, sex, and steroid use during treatment were not predictive of clinical outcomes. Consistent with the literature, MGMT promotor methylation was associated with improved OS (p=0.0103). CONCLUSION: Changes in circulating blood counts might serve as biomarkers of response to bevacizumab and overall outcome. The correlation of improved OS in patients with recurrent GBM and increases in platelet counts during bevacizumab therapy warrants further investigation and prospective studies.

HOUT-16. THE COST EFFECTIVENESS OF TUMOR TREATING FIELDS TREATMENT FOR PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA BASED ON THE EF-14 TRIAL

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BACKGROUND: With the EF-14 Trial reporting a 5-year survival rate of 13% for newly diagnosed Glioblastoma (GBM) patients treated with TTFields and maintenance temozolomide (TMZ) chemotherapy, a relevant number of patients will become long-term survivors. The impact on resource utilization from a health-economic standpoint is important for all health care decision-makers and therefore cost-effectiveness of TTFields treatment in first line GBM is assessed from a U.S. health system perspective. METHODS: The incremental cost-effectiveness ratio (ICER) for treatment of newly diagnosed GBM with TTFields and TMZ versus TMZ monotherapy was estimated. We used a 3-state area under the curve model including alive without progression, progressed disease, and death to simulate patient outcomes and a lifetime horizon for survival by integrating the 5-year Kaplan-Meier curves of the EF-14 trial with long-term GBM epidemiology data and U.S. background mortality rates. For the calculation of quality-adjusted life years (QALYs) health utility values reported by Garside et al. were used. Frequency of adverse events (AE) were based on the EF-14 trial. AE and supportive care cost estimates were derived from published literature. We applied a discount of 3% to future survival benefits and costs. Parametrical uncertainty was addressed by one-way and probabilistic sensitivity analyses. RESULTS: We calculated an undiscounted increase in mean survival of 1.8 life years for TTFields plus TMZ versus TMZ alone. After applying the 3% discount rate, the incremental total cost was $188,637. The incremental cost-effectiveness ratio (ICER) was $150,452 per life year gained and $197,336 per QALY gained. CONCLUSIONS: Mean lifetime survival and quality-adjusted survival substantially increases by treatment with TTFields plus TMZ compared to treatment with TMZ alone in newly diagnosed GBM patients. Treatment with TTFields for can be considered cost-effective within the reported range of willingness-to-pay thresholds in the United States based on the results of this analysis.

HOUT-17. ELDERLY PATIENTS >65YEARS OF AGE WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME GAIN LIFE TIME FROM TREATMENT WITH TUMOR TREATING FIELDS AND TEMOZOLOMIDE

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BACKGROUND: The EF-14 trial was the first randomized controlled trial to report a survival benefit for newly diagnosed GBM patients in more than a decade with a survival rate greater than 10% at 5-years. A post hoc subgroup analysis of the elderly (>65 years) EF-14 population showed the greatest survival benefit in this subgroup (HR = 0.51) with survival at 5-years of 15% for patients treated with TTFields and maintenance TMZ compared to no survivors in the TMZ alone arm. MATERIALS/METHODS: An integrated survival model was calculated, incorporating Kaplan-Meier data of the subgroup of over 65 years GBM patients from the EF-14 Trial with epidemiological data on long-term GBM survival. Future survival benefits were discounted at a rate of 3%. We also performed sensitivity analyses of the results and compared the results of our integrated survival model to regression based extrapolations of the trial data. RESULTS: Elderly patients treated with TTFields and TMZ achieved an undiscounted mean lifetime survival of 3.66 years compared to 1.32 years for patients treated with TMZ alone, resulting in a gain of 2.34 life years for TTFields plus TMZ. After application of the 3% discount rate for future survival benefits, the resulting life time survival is 3.04 vs. 1.29 life years respectively, resulting in 1.75 incremental life years gained. CONCLUSIONS: In the subgroup of elderly patients of the EF-14 Trial treatment with TTFields plus TMZ resulted in a substantial increase of the mean lifetime survival of 1,75 life years compared to treatment with TMZ alone, despite the older age of the patients with a naturally lower potential for long-term survival. These results together with the low incidence of adverse events in the EF-14 trial indicate that it may be appropriate to opt for TTFields treatment in newly diagnosed GBM patients 65 years and older.

HOUT-18. COST EFFECTIVENESS OF TREATING GLIOBLASTOMA PATIENTS AGE 65 YEARS OR OLDER WITH TUMOR TREATING FIELDS PLUS TEMOZOLOMIDE VERSUS TEMOZOLOMIDE ALONE

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BACKGROUND: TTFields plus TMZ treatment significantly increased five-year overall survival results compared to TMZ alone in all patients in the EF-14 trial, with the subgroup of patients age 65 and older showing the greatest survival benefit (hazard ratio 0.51 [CI 0.33–0.77]). This is the first report on the cost-effectiveness of TTFields in elderly GBM patients. METHODS: Patient outcomes were simulated using a 3-state area under the curve model. Survival was modelled over a lifetime horizon by integrating 5-year survival results of the EF-14 trial with long-term GBM epidemiology data and U.S. background mortality rates. Patient utilities from a previous publication were used to calculate quality-adjusted life years (QALYs). Adverse events of TTFields and TMZ were derived from the EF-14 trial for patients over 65 years, the costs from published literature. RESULTS: Elderly patients treated with TTFields and TMZ achieved mean lifetime survival of 3.66 years (3.04 years discounted). Patients treated with TMZ alone achieved a mean lifetime survival of 1.32 years (1.29 years discounted) The resulting incremental life years gained were 1.75 years discounted and the incremental quality-adjusted life years (QALYs) were 1.35. The incremental total cost was $192,000. The ICER was $109,500 per LYG and $142,400 per QALY gained. The probability of TTFields being cost-effective was 85% at a willingness-to-pay threshold of $200,000 per QALY. CONCLUSIONS: Treating newly diagnosed GBM patients over 65 years with TTFields and TMZ has the potential to increase mean lifetime survival and quality-adjusted survival substantially compared to TMZ alone. TTFields therapy, even when evaluated at its full list price, demonstrated high probability of cost-effectiveness at willingness-to-pay thresholds reported in economic literature for the United States. These results indicate that patients over age 65 may not only benefit from TTFields treatment, but also that their treatment may be cost-effective.

HOUT-19. TREATMENT PATTERNS, OUTCOMES, AND PROGNOSTIC INDICATORS IN ELDERLY PATIENTS WITH GLIOBLASTOMA: A RETROSPECTIVE SINGLE INSTITUTION ANALYSIS

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OBJECTIVE: There are limited evidence-based guidelines for treatment of elderly glioblastoma (GBM) patients. This analysis explores prognostic indicators that may help guide treatment in this population. METHODS: This retrospective analysis includes 106 patients 75 years old seen at the Preston Robert Tisch Brain Tumor Center between 2004–2016. Kaplan-Meier methods estimated overall survival (OS). Time to OS was censored at last follow-up for patients still alive. Univariate Cox proportional hazards models assessed predictors of OS. RESULTS: Median age at diagnosis was 79 years (range: 75–93). Median follow-up was 68.8 months. Median survival was 8.1 months (95% CI: 6.5, 9.3). Patients with biopsy-only had significantly worse OS than patients with gross total resection (GTR) (Hazard Ratio (HR)=2.64 (95% CI: 1.69, 4.12); p<0.001) or subtotal resection (STR) (HR=2.54 (95% CI: 1.33, 4.85); p=0.005; OS=5.0 vs 9.8 vs 12.7 months, respectively). Of 84 patients with data available regarding radiotherapy (RT), there was no significant difference in OS between patients receiving 3 weeks of RT versus 6 weeks. Patients not receiving RT had significantly worse OS than patients with 3-week (HR=7.66 (95% CI: 3.25, 18.03); p<0.001) or 6-week courses (HR=8.95 (95% CI: 4.16, 19.27); p<0.001; OS=4.5 vs 9.2 vs 11.2 months, respectively). Patients with biopsy-only tended to have lower KPS and were less likely to receive RT. In the subset of 66 patients with known methylation status, unmethylated patients showed a trend towards worse OS than methylated patients (OS=7.5 vs 11.5 months, respectively; HR=1.65 (95% CI: 0.987, 2.759); p=0.056). A post-op KPS80 was predictive of better OS (9.3 vs 5.4 months; HR=0.976 (95% CI: 0.954, 0.999); p=0.043). CONCLUSIONS: Biopsy-only, no RT, lower KPS and absence of MGMT methylation were associated with worse OS. Extent of resection (STR vs GTR) and duration of radiation (3 weeks vs 6 weeks) did not influence OS.

HOUT-20. AN ONLINE CALCULATOR FOR THE PREDICTION OF SURVIVAL AND ADJUVANT TREATMENT BENEFIT IN GLIOBLASTOMA PATIENTS

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BACKGROUND: Although survival statistics for glioblastoma patients are well-defined on population-scale, individualizing survival prognostication remains challenging. Furthermore, treatment efficacy proven on group-level does not directly apply to the same extent in each individual patient. This study compares a variety of statistical and machine learning algorithms in their ability to predict the individual survival benefit of adjuvant chemo- and radiotherapy. The best performing model was used to develop an online survival calculator. METHODS: Patients operated for a histopathologically confirmed glioblastoma were extracted from the Surveillance Epidemiology and End Results database (2005–2015) and split into a training and hold-out test set with an 80/20 ratio. Fifteen statistical and machine learning algorithms were trained based on 13 demographic, socioeconomic, clinical, and radiographic features to predict overall survival, one-year survival status, and subject-level ten-year survival curves. The resultant models were compared based on discriminatory performance and calibration, as well as interpretability, predictive applicability, and computational efficiency. FINDINGS: In total, 20,821 patients met our inclusion criteria. The accelerated failure time (AFT) model demonstrated superior performance in terms of discrimination (concordance index 0.70), calibration, interpretability, predictive applicability, and computational efficiency compared to cox-proportional hazards regression and a variety of competitive machine learning models. The AFT model was, therefore, deployed through a free, publicly-available software. INTERPRETATION: This study provides a framework for the development of outcome prediction tools in cancer patients, as well as an online calculator for the prediction of survival and treatment benefit in glioblastoma patients. Model deployment of survival calculators requires a multidimensional assessment rather than a single metric comparison. Future research efforts should improve the interpretability, predictive applicability, and computational efficiency of existing machine learning algorithms, increase the granularity of population-based registries, and externally validate the proposed prediction tool.

HOUT-21. REAL-WORD EVALUATION OF THE IMPACT OF RADIOTHERAPY AND CHEMOTHERAPY IN ELDERLY PATIENTS WITH GLIOBLASTOMA BASED ON AGE AND PERFORMANCE STATUS: A NATIONAL CANCER DATABASE ANALYSIS

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OBJECTIVES: This retrospective study evaluates the impact of Karnofsky performance status (KPS) in addition to age on the management and outcomes of elderly patients with glioblastoma (GBM). METHODS: The National Cancer Database (NCDB) was queried between 2004–2015 for GBM patients with age 60 and reported KPS. Four groups were created: Age 60/ KPS<70 (Gp1), Age 60–69/KPS 70 (Gp2), age 70–79/KPS 70 (Gp3), age 80/KPS 70 (Gp4). Univariate (UVA) and multivariable (MVA) modeling with Cox regression determined predictors of survival (OS), and propensity score-matched analysis was performed. RESULTS: A total of 30,530 patients were included. Median age at diagnosis was 69, and median follow-up was 7.3 months. Median survivals were 5.2, 11.3, 6.3 and 3.7 months in groups 1, 2, 3 and 4, respectively (p <0.001). Combined chemotherapy (CT) and radiotherapy (RT) was received in 65.5% of Gp2, 53.7% of Gp3, 48.2% of Gp1 and 33.1% of Gp4 (p<0.001). On both UVA and matched OS analysis, all patients benefited from combined CT and RT, except those in Gp1, who did better with CT alone (p<0.01). RT alone was associated with the worst OS in all groups (p<0.01). Predictors of worse OS on MVA were greater age, lower KPS, white ethnicity, higher comorbidity score, worse socioeconomic status, treatment in a community center, tumor multifocality, subtotal resection, non-IMRT use, and no adjuvant treatment (all p<0.01). CONCLUSIONS: Based on this NCDB review, patients with GBM aged 60 with poor KPS have better survival following CT alone, whereas elderly, even those 80 years old, with good KPS fared best with combined CT and RT. RT alone resulted in worse OS compared to CT or combined CT and RT. Functional status is an independent, key prognostic factor that should be incorporated with age for management decisions in the elderly GBM population.

HOUT-22. TWO CASE REPORTS OF PATIENTS WITH SUPERIOR SEMICIRCULAR CANAL DEHISCENCE AND EHLERS-DANLOS SYNDROME

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BACKGROUND: Superior semicircular canal dehiscence (SSCD) is a rare bony defect that results in an opening between the inner ear and middle cranial fossa, which often results with patients presenting with auditory and vestibular symptoms. Ehlers-Danlos Syndrome (EDS) is an inherited connective tissue disorder that is characterized by joint hypermobility and skin extensibility. CLINICAL PRESENTATIONS: We previously reported the case of a 50-year-old woman with a 15-year history of auditory and vestibular symptoms. Computed tomography confirmed the presence of bilateral dehiscence in her superior semicircular canals. Past medical history was significant for Ehlers-Danlos Syndrome (EDS) Hypermobility Type. We present a new case presentation of a 36-year-old female with similar symptoms and a medical history significant for EDS-Hypermobility and confirmation of bilateral superior semicircular canal dehiscence. DISCUSSION: Both patients were treated via middle fossa craniotomy, with follow-up visits indicating symptom improvement. Because of their rarity, this study highlights the possibility of a connection between the two diagnoses.

HOUT-23. ASSOCIATION BETWEEN TREATMENT FACILITY VOLUME AND MORTALITY IN PATIENTS WITH GLIOBLASTOMA (GBM): A LARGE NATIONAL ANALYSIS

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BACKGROUND: GBM is an aggressive and incurable primary malignancy of the brain, treated with surgical resection and chemo-radiotherapy, yet it has a dismal prognosis of 12–14 month overall survival (OS). Optimal outcomes require an experienced team providing multidisciplinary management. We explored the association of treatment facility volume and mortality in patients with GBM. METHODS: We identified incident GBM (ICD-O-3 code: 9440/3) cases from the National Cancer Database (NCDB) (2004–2013) and utilized Cox-regression to determine the facility volume-outcome (volume=quartiles; Q) relationship, adjusting for year of diagnosis, demographic (sex, age, race, ethnicity), socio-economic (income, education, insurance type), geographic (area of residence, treatment facility location, travel distance) and co-morbidity factors (Charlson-Deyo score). RESULTS: There were 114,467 patients (median age 60 years, range: 18–90) with GBM treated at 1207 facilities of which, 54.8% were men. Median annual facility volume was 5 patients/year (range: 0.1–136.4). The top 14 (1.2%) facilities treated >60 patients/year (10%). Median overall survival (OS) was 15 months There were significant differences (all $p<0.001$) in patient characteristics by facility volume. Unadjusted median OS by facility volume (months) was Q1: 29.1, Q2: 32.9, Q3: 36.4, Q4: 48.2 ($p<0.0001$). Multivariate analysis showed facility volume to be independently associated with all-cause mortality (Reference Q4; Q3 HR: 1.30, 95% CI 1.28–1.33; Q2 HR: 1.36, 95% CI 1.36–1.43; Q1 HR: 1.58 95% CI 1.50–1.67). OS disparity by facility volume is persistent but not worsening in recent years (2010–2013 vs 2004–2005). CONCLUSIONS: In GBM, facility-volume independently affects OS of the patients. Attempts should be made to address modifiable factors and get patients access to high-volume centers earlier in the disease course.

HOUT-24. DURAMATRIX-ONLAY® PLUS IN CRANIAL SURGERY IS ASSOCIATED WITH AN ACCEPTABLE COMPLICATION PROFILE: A CASE SERIES

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BACKGROUND: DuraMatrix Onlay® Plus (Stryker, Kalamazoo, Michigan) is a collagen dura membrane derived from purified bovine Achilles tendon. The matrix provides a scaffold for collagen synthesis and is intended to be used as an onlay without the need for dural sutures. OBJECTIVE: To describe our experience with 33 consecutive patients who underwent a duraplasty procedure using the novel DuraMatrix Onlay® Plus collagen dura membrane. METHODS: This is a retrospective case series of consecutive patients who underwent a duraplasty procedure at a single academic hospital in Los Angeles, California between May 2016 and March 2017. The primary outcome is the rate of cerebrospinal fluid (CSF) leak. Secondary outcomes include the rates of dural substitute complication, infection, and removal. RESULTS: Thirty-three patients underwent a duraplasty procedure using the DuraMatrix Onlay® Plus material. The average age of the patients was 41 ± 21 years (range 2–75 years). There were 18 females and 15 males. The majority of procedures were elective and for resection of a lesion (n = 19, 58%), with the average size of material used measured at 18 ± 14 cm2. There were no secondary CSF leaks at an average follow-up of 3 months. The rates of dural substitute complication, infection, and removal were 6%, 6%, and 3%, respectively. In one patient, the dural substitute was removed for concern of infection. CONCLUSION: DuraMatrix-Onlay® Plus is associated with an acceptable complication profile, including a low rate of CSF leak.

HOUT-25. ADHERENCE TO TUMOR TREATING FIELDS IN PATIENTS WITH HIGH-GRADE GLIOMA – A SINGLE CENTER EXPERIENCE

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BACKGROUND: The addition of alternating electric fields with low intensity (1–3 V/cm) and intermediate frequency (100–300 kHz), known as Tumor Treating Fields (TTFields), to adjuvant temozolomide demonstrated significant clinical benefits in newly diagnosed glioblastoma (GBM) patients. Post-hoc analysis of the EF-14 trial revealed a strong correlation of adherence to TTFields therapy with prolonged OS, underlining the importance of a high compliance rate. Here, we report on TTFields therapy adherence of thirty-four patients with high-grade glioma (HGG) treated with TTFields. METHODS: Thirty-four patients diagnosed with GBM (28 patients) and astrocytoma WHO°III (6 patients), respectively, were treated with TTFields at our institution. Patients were introduced to the therapy during our neurooncologic consultation hours. We evaluated the compliance reports that were generated at the monthly technical check of the device. RESULTS: The median age of high grade glioma (HGG) patients at therapy start was 53.5 [32–67] years. 21 of the 28 GBM patients were newly diagnosed and the remainder had recurrences. These patients showed a gender distribution female to male of 1:1.15, demonstrating a higher ratio of female patients compared to the typical GBM population with 1:1.7. Patients with newly diagnosed GBM were on TTFields therapy for a median of 6.5 months with median treatment compliance of 86.4%. No significant difference regarding compliance was observed between female (87.1%) and male (81.7%). Comparison of patients with newly diagnosed GBM and recurrent GBM showed no significant difference in therapy adherence with a median compliance of 80.0%. Astrocytoma WHO°III patients showed a compliance rate of 86.2%. CONCLUSION: TTFields therapy was well accepted by high grade glioma patients treated at our institution with a high median compliance to the therapy substantially above the recommended 75% threshold and irrespective of sex and diagnosis.

HOUT-26. SURVIVAL OUTCOMES IN GLIOBLASTOMA PATIENTS USING TTFIELDS: THE BAYLOR SCOTT & WHITE MEDICAL CENTER IN CENTRAL TEXAS EXPERIENCE

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BACKGROUND: Glioblastoma is a CNS cancer with extremely poor survival rates despite advances in chemoradiation therapy. TTFields (Optune) are an FDA approved option for treatment of glioblastoma. PURPOSE: This article aims to determine whether Optune compliance rates in patients with both newly diagnosed and recurrent glioblastoma affects survival. METHODS/ANALYSIS: A comprehensive list of glioblastoma patients was extracted from the Baylor Scott & White tumor registry from 2012–2018. Data on compliance, demographics, IDH/MGMT status and survival was compiled on Optune users. These variables were analyzed using the Welch T-test to ascertain their impact on compliance and survival. A Pearson correlation coefficient was calculated to determine the relationship between compliance and survival post-Optune. RESULTS: 37 patients were identified (median age: 60, range: 21–86). Males were more affected (60.0%), the frontal lobe was the most common site (35.1%), and the median survival was 17 months. There was no statistical difference in survival length (months) based on gender (males = 21.5, females = 18.9, $p = 0.52$), MGMT status (methylated = 21.8, unmethylated = 17.8, $p = 0.40$), and IDH status (wild = 19.1, mutated = 30.2, $p = 0.41$). The average Optune compliance was 66.2%, with no statistical difference based on age (age < 50 = 54.1%, age > 50 = 71.1%, $p = 0.06$) and gender (male = 66.6%, female = 65.6%, $p = 0.89$). The correlation coefficient comparing compliance and survival post-Optune was $R = -0.247$ ($p = 0.141$). CONCLUSION: Studies have shown that Optune compliance of 75% or greater can improve outcomes. In our own limited study, the average compliance was markedly below at

66.2%, with no impact on the median 17-month survival. Age and gender did not play a role in compliance rates. This illustrates that further education is required in central Texas to improve compliance and patient outcomes.

HOUT-27. THE CHALLENGE OF HEALTH UTILITY VALUES FOR GLIOBLASTOMA PATIENTS WITH LONG-TERM SURVIVAL

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BACKGROUND: The EF-14 trial was the first randomized controlled trial to report a GBM survival rate greater than 10% at 5-years. The quality-adjusted life year (QALY) has been developed to estimate the economic impact of new treatments. For the assessment of QALYs, health-utility values are needed. Utilities are preference weights that are determined either through direct elicitation methods or generic preference-bases measures, however in oncology they do not account for patients with long-term survival. This approach may understate QALYs of long-term survivors, as patients alive 5-years after diagnosis will be assigned the same utility value as patients recently diagnosed. METHODS: We performed a comprehensive review of the published literature regarding health-utility values in GBM patients through a Pubmed search using glioblastoma AND health utility OR glioblastoma AND health utilities OR glioblastoma and health preference. RESULTS: A total of 77 publications were screened with only 3 relevant publications in health-economic context. All three publications use utilities for GBM derived from the same source (Garside et al.). These utilities were obtained from the NHS Value of Health Panel (VoHP) of which 36 members rated a total of 9 descriptive health-state scenarios. This approach assumes the constant presence of adverse events during the respective state and a constant decline in quality of life of patients in progressive state, which may particularly be inadequate for long-term survivors with sustained stable disease. CONCLUSIONS: Health economic evaluations of GBM treatments use utilities from Garside. There are no published utilities for GBM in a general population sample, adequately adjusting the utilities with time from diagnosis. Deriving and validating health preference-based measures from existing condition-specific questionnaires (e.g. the QLQ C-30 with its brain cancer module BN20) specifically for GBM would be beneficial and allow for adjusted utility values with time from diagnosis as already developed in multiple myeloma.

HOUT-28. CLINICAL EXPERIENCE WITH TUMOR TREATING FIELDS (TTFIELDS, OPTUNE®) IN ISRAEL - PATIENT ACCEPTANCE AND SAFETY

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BACKGROUND: TTFields are low intensity, intermediate frequency alternating electric fields, delivered through a portable, non-invasive device (Optune, Novocure™). The EF-14 phase III trial showed significant prolongation of both progression-free and overall survival in newly diagnosed glioblastoma (GBM) patients. This is the first report on clinical experience with 110 patients treated with TTFields in Israel, focusing on safety and patient acceptance in our country. METHODS: Patients received Optune-prescription in 6 neuro-oncology centers in Israel and used the device for at least one month. The cohort included both newly diagnosed (n=53) and recurrent GBM (rGBM, n=57) patients. The male/female ratio was 74/36 with a median age of 58.0 (18–82) years at start of treatment. Of patients with rGBM, 51% were treated at first progression. RESULTS: Our data shows a high acceptance, with a compliance rate (monthly usage of the device) within the first 3 months of 80% for newly diagnosed GBM and 66% for rGBM. Median treatment compliance was independent of age, sex and stage of disease. In addition, our data indicates that the compliance is not negatively correlated with time on treatment, as the median compliance for newly diagnosed GBM patients was still at 79% within the first 6 months. With regards to device-related side effects, data shows that in total 30 patients (27%) experienced mild-moderate skin irritations that were usually manageable with local steroid creams and did not cause significant treatment breaks despite the warm climate in Israel. Other side effects related to the device were heat sensation in 10 patients (9%) and electric sensation in 9 patients (8%) that did not cause significant treatment interruption. CONCLUSIONS: The accumulating clinical experience shows that TTFields are well-tolerated by patients in Israel without any major deviation from reported treatment-related side effects. Treatment compliance remains stable during a 6-month period.

HOUT-29. HEALTHCARE COSTS FOR HIGH-GRADE GLIOMAS: A POPULATION-BASED STUDY

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Clinical management of patients with high-grade gliomas (HGGs) is very challenging both clinically and financially. As costs of cancer care in the US are expected to increase and oncology care is switching from volume-based to value-based pattern, efforts are needed to establish effective and efficient management of patients with HGGs. However, relevant data are limited. In this study, we retrospectively identified 88 primary HGGs patients diagnosed and treated at our institution between January 1, 2011 and February 28, 2017 and linked patient clinical information from electronic health record with all insurance claims data (all paid) from Excellus BCBS. Among these patients, the median age at diagnosis was 59 years and the majority of the patients were white (94.3%) with GBM (80.7%). Total median healthcare costs for clinical management of HGGs were $184,159.83 (95% CI: $151,214.98, $222.431.36). The largest component of healthcare costs was outpatient service, followed by inpatient costs. When we examined costs by service type, we found the leading cost was radiology service, followed by inpatient surgical, prescription drugs, inpatient medical and outpatient pharmacy. Compared with patients under non-commercial insurance, patients under commercial insurance had longer survival time (median: 411 days vs. 358 days, $p = 0.563$), higher healthcare costs in total ($235,732.85 vs. $142,134.07, $p < 0.001$), and in each phase of clinical care. We further observed a U-shaped curve healthcare costs pattern, i.e., healthcare costs were high in the phase of initial care (3-month after diagnosis) and 9-month after initial diagnosis with relative low between these two phases. Generalized linear model showed patients with commercial insurance, better Karnofsky Performance Status, longer survival time had higher healthcare costs. Our real-world study demonstrated that healthcare costs for patients with HGGs were substantial and such high healthcare costs were positively associated with patient survival and commercial insurance.

HOUT-30. TUMOR TREATING FIELDS (TTFIELDS) IN COMBINATION WITH LOMUSTINE (CCNU) AND TEMOZOLOMIDE (TMZ) IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM)

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INTRODUCTION: TTFields in combination with TMZ showed significant survival benefit for newly diagnosed glioblastoma patients in the EF-14 trial, compared to TMZ standard therapy; independent of MGMT-promotor methylation-status, age and performance status. Recently, the CeTeG trial (NOA-09) demonstrated improved efficacy of lomustine (CCNU)/TMZ compared to TMZ standard therapy in newly diagnosed MGMT-promotor methylated GBM patients. Given that TTFields demonstrated a strong safety profile as well as a high potential being combined with other modalities and the very promising results for GBM patients with methylated MGMT-promotor in the CeTeG trial, there is a strong rationale in combining these modalities. This is the first report on patients, treated with a combination of both treatment regimens, TTFields and CCNU/TMZ. METHODS: Patients with newly diagnosed GBM and MGMT-promoter methylation were treated after surgery and radiochemotherapy with a combination of TTFields plus CCNU/TMZ at our institutions. Patients with complete resection (N=4), partial resection (N=3) as well as biopsy (N=1) were included in the analysis. We assessed safety and feasibility of this combined therapy. RESULTS: Currently, eight patients (medians: age 49, [39–69]; KPS 90, [70–100]) have been treated and analyzed: GBM IDH mutant (N=2), IDH wild-type (N=6). To-date, CTCAE grade 3 hematotoxicities were observed in two patients, but were not considered to be related to the addition of TTFields to CCNU/TMZ. No further non-hematologic toxicity was observed. In one patient a

grade 3 event (herpes zoster) emerged. Regarding medical device site reactions, low-grade skin irritations were detected in four patients (50%), corresponding to the safety analysis from the EF-14 trial with low-grade skin irritations in 52% of the patients. CONCLUSION: These data provide first indications that the combination of TTFields and CCNU/TMZ is probably safe and feasible. Additional follow-up and a higher sample size are needed for further toxicity analysis and efficacy assessment of this combination.

HOUT-31. CARE PATTERNS AND TREATMENT EFFICACY: A CLINICAL SERIES OF PRIMARY GLIOBLASTOMA WITH AN EMPHASIS ON OLDER ADULTS

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INTRODUCTION: Research suggests that glioblastoma (GBM) patients enrolled in clinical trials have better-than-average prognosis. However, since implementation of the Stupp protocol and approval of bevacizumab as second-line treatment, there have been limited reports of outcomes-related data for GBM patients in a real-world, multidisciplinary clinical setting. We report here care patterns and outcomes in patients with GBM in a real-world setting. METHODS: From October 2015 to March 2018, 47 patients with primary GBM treated at Abbott Northwestern Hospital were enrolled in this study. Demographic, pathologic, and clinical information was abstracted from electronic medical records. Quality of life was measured at specified time-points during treatment with the MDASI-BT questionnaire. Additional data analysis was performed for a subgroup of ≥65 year old patients. RESULTS: Median patient age was 58 years (range, 23–80) and 25.5% were aged ≥65 years. Gross total tumor resection was achieved in 91.5% patients and a majority of the patients received 6 weeks of chemoradiation post-surgery. Patients received adjuvant temozolomide for a median of 7 cycles (range, 2–18). MGMT-methylation was observed in 32% cases. Second-line therapy consisted of bevacizumab with/without carboplatin in 63.2% patients and 15 patients received TTFields treatment. Median progression-free survival (PFS) in this series was 8.9 months in all patients and 9.6 months in patient aged ≥65 years. Median overall survival (OS) was 22.4 months for both the full-cohort and elderly subgroup. Patients with MGMT-methylated tumors had a longer median PFS and OS, but the differences were not statistically significant. Three or more moderate to severe treatment related symptoms were observed in 51.8% patients. CONCLUSION: PFS and OS in this real-world setting were comparable to outcomes noted in controlled clinical trials. These results indicate that favorable survival rates in GBM patients, including elderly individuals, may be achieved with a multidisciplinary treatment approach.

HOUT-32. SIGNIFICANCE OF COMORBIDITY INDEX IN ELDERLY PATIENTS WITH GLIOBLASTOMA

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BACKGROUND: Comorbidity is a common problem in the treatment of elderly patients, but there is no standard method of the evaluation in glioblastoma patients. Gagne comorbidity index (GCI) has been proposed to be an integrated prognostic index of the Charlson comorbidity index and Elixhauser index, associating with the short-term survival of various diseases. We examined the relationship between GCI and prognosis of elderly patients with glioblastoma. METHODS: 31 patients over 70 years treated at our hospital from Dec.2006 to Aug.2017. 16 males and 15 females. Mean age 76.8 years (70–86 years old). Relationship to overall survival (OS) was examined focusing on the age, GCI at hospitalization(low:≤1, intermediate:2–4, high:≥5), Karnofsky Performance Status (KPS), surgery, radiotherapy (standard and hypofractionated), and temozolomide-based chemotherapy. RESULT: Median OS of entire cohort was 13.0 months. There was no significant correlation between age and GCI, but a negative correlation between KPS and GCI (r=-0.427). Median OS was longer in the resection group (resection; 13.5m vs non-resection; 8.6m), and in chemo-radiotherapy (standard radiotherapy+TMZ: 13.5m and hypofractionated radiotherapy+TMZ: 10.1m), though, GCI has no relationship to such treatment option. Nevertheless, high GCI indicated poor prognosis with a median OS in the group of low GCI:13.0m, intermediate GCI:14.5m and high GCI:5.3m (p=0.002). DISCUSSION: The presence of comorbidities is one of the factors related to prognosis, and glioblastoma affecting the brain is in a disadvantageous condition due to cognitive dysfunction and paralysis. Since surgical excision and radiation chemotherapy lead to improvement in prognosis even in the elderly, it is important to complete the treatment paying maximum attention to the management of comorbidities. GCI would be useful to evaluate comorbidities. Further examination is needed in large cohort.

HOUT-33. FUNCTIONAL OUTCOME OF GLIOMA SURGERY UNDER AWAKE CONDITION AIMED FOR PRESERVATION OF SEVERAL FUNCTIONAL DOMAINS

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BACKGROUND: Awake surgery for the eloquent cortex is common strategy for glioma surgery. Although a recent emphasis has been placed on awake surgery for both dominant and non-dominant cerebral hemispheres to preserve neurological/neuropsychological functions, those functional outcomes are not well investigated since few studies focused on longitudinal recovery process. OBJECTIVE: This study explored the outcome of neurological/neuropsychological functions following awake surgery until chronic phase. METHODS: A total of 87 patients with glioma who underwent awake surgery between September 2013 and September 2017 were included, and of these 66 patients matched our inclusion criteria (age: mean, 46.0; range, 16–73). Each patient was assessed for neurological/neuropsychological functions including sensorimotor function, naming, working memory, low- and high-level mentalizing and visuospatial cognition at before surgery, as well as acute and chronic phase. Additionally, scores for the Karnofsky Performance Status (KPS) were collected. RESULTS: Almost all neurological/neuropsychological functions recovered within 3 months postoperatively, even when transient deficits were observed in the acute phase. However, deep sensory perception deficits and visuospatial cognitive disorders persisted into the chronic phase. Disorder probabilities at chronic phase were as follows: deep perception disorder, 15.4 % of patients with parietal lesions; visuospatial cognition, 14.3% of patients with right hemisphere. KPS score ≥ 90 was achieved in 86.0 % of patients with lower grade glioma, whereas only 52.2% of glioblastoma patients scored ≥ 90. Primary causes of declined KPS were disorder of visuospatial cognition, sensorimotor function including deep sensation, aphasia, and emotional function, which influence on their professional work and household work. CONCLUSIONS: Awake surgery leads good functional outcome at chronic phase on neurological/neuropsychological functions, except for deep sensory and visuospatial cognition. Since sensation and visuospatial cognitive disorder make major impact on patients' independence level, further importance should be placed on preserving these functions during surgery.

HOUT-34. ADVERSE EVENTS IN BRAIN TUMOR SURGERY: INCIDENCE, TYPE AND IMPACT ON CURRENT QUALITY METRICS

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OBJECT: The aim of the study was to determine pre-operative factors associated with adverse events occurring within 30 days after neurosurgical tumor treatment. METHODS: All adult patients that were hospitalized due to a benign or malignant brain tumor in our department between 2013 and 2017 were retrospectively assessed for quality indicators and adverse events. Administrative data were obtained, the age-adjusted Charlson Comorbidity Index (CCI) was calculated and logistic regression analyses were performed to determine risk factors for adverse events. Detailed analyses of all readmissions and reoperations were performed. RESULTS: Overall, 2511 cases were enrolled. The most common diagnosis was glioma (42%). The 30-days unplanned readmission rate was 5.7%. The main reason for readmission was tumor progression. Every 10thpatient had an unplanned reoperation. The incidence of surgical revisions due to infections was 2.3%. Taking together all monitored adverse events, male patients had a higher risk for any of these complications (OR: 1.236, 95%CI: 1.025–1.490, p=0.027). Age, sex and histological diagnosis were predictors of experiencing any complication. The CCI was significantly higher in patients that were readmitted within 30 days (p= 0.045) and correlated with a higher 30-days mortality rate (p< 0.001), a higher nosocomial infection rate (p< 0.001), a higher SSI rate (p= 0.027) and a longer length of stay (p< 0.001) in univariate analysis. CONCLUSIONS: We found that most predictors of adverse events and outcome rates are based on preoperative underlying medical conditions of the patient and are not modifiable by the surgeon. Comparing our results to the literature, we conclude that differences in readmission and reoperation rates are strongly influenced by standards in surgical decision making and that comparison of outcome rates between different health-care providers on an international basis is challenging. Each health-care system has to develop own metrics for risk adjustment and assessment of outcome variables, that require regular reassessment.

HOUT-35. RETROSPECTIVE ANALYSIS OF OUTCOMES IN HOSPITALIZED MALIGNANT BRAIN NEOPLASM PATIENTS WITH STATUS EPILEPTICUS

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OBJECTIVE: To investigate the key characteristics and outcomes in patients with Malignant Brain Neoplasm and Status Epilepticus. BACKGROUND: Epileptic seizures are a major comorbidity in patients with malignant brain neoplasm (MBN), either at presentation or as a sign of progression. Seizures are more challenging to control in this patient population, especially with rapidly growing tumor and often progress to status epilepticus (SE). We conducted this study to investigate the key characteristics and outcomes in patients with MBN and SE. Methods: Analysis of National Inpatient Sample data (2003–2013) showed a total of 76,357 patients discharged with a primary diagnosis of MBN, ICD-9 Code 191.X. Among this patient population, 557 patients with SE were identified. We compared age, length of stay (LOS) and total hospital charges between MBN patients with and without SE, as well as the association between MBN and SE with patient race, sex, and disposition status. RESULTS: Analyses revealed that 0.7% of MBN patients were found to have SE. No significant difference was found in age at admission of patients with SE (50.69 vs 51.76 p=0.233). SE was associated with African American (AA) race as compared to non-AA (1.4% vs 0.7%), longer LOS (11.54 vs 6.92 days), higher hospital charges ($113,416 vs $65,159), disposition other than home (69.9% vs 44.7%) and increased mortality (15.5% vs 4.1%). CONCLUSION: The results suggest SE is associated with increased morbidity and mortality amongst MBN patients. Our results show an association between SE and increased LOS, worse disposition, higher hospital charges, and mortality. Therefore, early identification and aggressive treatment of SE is warranted in hospitalized patients with Malignant Brain Neoplasm. Future prospective studies are needed to investigate different treatment approaches for Status Epilepticus in hospitalized patients with brain tumors.

HOUT-36. INSTITUTIONAL COMPLIANCE WITH TUMOR TREATING FIELDS FOR GLIOBLASTOMA

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Tumor Treating Fields (TTFields) is a relatively new therapeutic intervention, which uses alternating electric fields to deliver low intensity, intermediate frequency current to solid brain tumors, thereby inhibiting mitosis. In the EF11 Phase III study in recurrent glioblastoma patient compliance, stratified by over or under 18 hours/day, for 28 days conferred a variable median survival benefit of 7.7 vs 4.5 months. In this study we report our institutional compliance in efforts to elucidate long term, >28 day, patient compliance in a clinical setting. After IRB approval we reviewed eleven patient TTFields compliance histories with recurrent and newly diagnosed GBM (age mean 55 ± 9 years). All patients underwent resection, chemotherapy (temozolomide +/- bevacizumab), and radiation therapy prior to TTField treatment. Duration of device use, hours of use per day, and reason for device discontinuation were reviewed with time to progression (TTP) determined by RECIST criteria. 6 patients met the recommended compliance of >18 hours per day (average 19.7 ± 1.0 hours/day), while 5 patients did not (averaging 11 ± 5 hours/day). 10 of 11 patients used TTFields for more than 100 consecutive days, with an average of 165 ± 85 days (range = 50 to 426 days). Patients discontinued use for a variety of reasons; death, insurance issues, and transition to hospice services following disease progression. After the start of TTFields therapy, the median TTP was 5.5 months (range 2 to 39 months). 5 patients with the longest TTP (>6 months) averaged >19.5 hours/day of treatment. While, 4 patients with the shortest TTP (3 months) averaged 11 hours/day. In a traditional clinical setting, many patients maintained compliance of >18 hours/day for an average of 165 days. In summary, we demonstrate that outside the scope of a clinical trial, high compliance with this device is a realistic and achievable goal.

IMMUNOLOGY

IMMU-02. ONCOLYTIC HSV THERAPY ENHANCES GLIOBLASTOMA CONTROL VIA THE EXPANSION OF FUNCTIONAL TUMOR-SPECIFIC T CELLS AND MODULATION OF MYELOID CELL POPULATION

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Immunotherapeutic approaches to treating glioblastoma (GBM) such as immune checkpoint blockade or dendritic cell vaccines have so far failed in recent clinical trials, despite showing some success in other cancer types. This failure has been attributed partly to the profound immunosuppressive tumor micro-environment (TME), characterized by dysfunctional cytotoxic T cell response and significant infiltration of regulatory lymphocytes and suppressive myeloid cells. We hypothesize that an oncolytic HSV1 (oHSV) which has a direct tumor cytotoxicity and potent *in situ* immunostimulation may reverse these immune dysfunction state and therefore improve tumor regression. Using several unique reagents, we found that oHSV treatment led to substantial reduction in tumor volume compared to PBS-control mice (oHSV- 9/11 vs PBS- 0/5; p= 0.0022), translating to longer term survival. Interestingly, oHSV led to improved tumor-specific T cells response with an expansion of tumor antigen-specific CD8+ T cells within the TME on both day 3 (oHSV- 4% vs PBS- 2%; p = 0.02) and day 7 (oHSV- 6.51% vs PBS- 1.33%; p = 0.04) following treatment, and a higher expression of IFNγ by CD8+ T cells compared to control (oHSV- 0.21% vs PBS- 5.60%; p = 0.04), suggesting an improved tumor-specific T cell functionality. Importantly, we found that this expansion of tumor-antigen specific CD8+ T cells inversely correlated with the tumor volume (R^2= 0.41, p= 0.008). oHSV also led to a decrease in microglia and suppressive myeloid cell population which correlate negatively with the number of infiltating tumor-specific CD8+ T cells, suggesting they may play a role in modulating oHSV-mediated expansion of tumor specific T cells. Overall, using these novel tools, we demonstrate for the first time, that oHSV, in addition to its direct cytotoxic effect, play an important immunostimulating role through its modulation of tumor-specific T cells and myeloid cell population within the GBM tumor microenvironment.

IMMU-03. DEVELOPMENT OF CYTOMEGALOVIRUS (CMV) BASED DNA VACCINES FOR GBM USING THE UNITE PLATFORM

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Glioblastoma (GBM) remains an aggressive, deadly disease in brain cancer, with the median survival of 15 months remaining unchanged for decades. Using our investigational UNITE platform, we are developing a DNA vaccine using CMV antigens. The UNITE platform is based, in part, on a lysosomal targeting technology which can result in increased antigen presentation, a balanced T cell response, and subsequent immunologic benefit. The presence of CMV proteins in GBM offers a unique opportunity to specifically target tumor cells, unlike other cancers. This targeting approach, in the vaccination of mRNA transfected autologous dendritic cells, was employed by Drs. John Sampson and Duane Mitchell, at Duke University in a Phase I trial. Additionally, an ongoing phase II trial, run by Dr. Mitchell at University of Florida, is leveraging the same targeting approach. Promising results were observed using pp65 (a CMV structural protein) loaded dendritic cells in the phase I clinical trial (Clinical Cancer Research, 2014, Pg-2684). Our objective to develop the DNA vaccine is to achieve both a lower cost and quick turnaround time by eliminating need to isolate and culture autologous dendritic cells and to explore other ways of vaccine improvement. We have tested CMV antigens with UNITE platform in mice using intradermal injection followed by electroporation and found robust T cell response. We are also developing orthotopic GBM model in syngeneic mice and to closely mimic human GBM where tumor cells express CMV proteins, we have made stable murine glioma cell lines expressing pp65 or pp65 and gB fused with luciferase as reporter. Therapeutic studies are ongoing in the intracranial GBM mouse models. We believe that if successful, DNA vaccines using CMV proteins as targets, either alone or in combination with other therapies, will be a powerful and cost effective treatment to fight this dreadful cancer.

IMMU-04. OVERCOMING IMMUNE EVASION IN GLIOBLASTOMA

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The negative phase 3 trial of anti-PD-1 in human glioblastoma (GBM) contradicts preclinical data showing eradication of GL261 mouse brain tumors by checkpoint inhibitors (CPI). To understand whether the tumor microenvironment contributed to this discrepancy, we immunoprofiled 19 human GBM tumors by CyTOF mass cytometry. We observed a macrophage-rich, T cell-poor microenvironment, with low PD-L1 expression. In contrast, GL261 tumors showed substantial T cell infiltration and high PD-L1 expression. Seeking a model that more accurately reflects the immune landscape of human GBM, we immunoprofiled SB28, a C57BL/6-derived transplantable model of GBM driven by PDGFB, $NRAS^{G12D}$, and p53 loss. CyTOF analysis of SB28 revealed a macrophage-rich, T cell-poor, PD-L1-low microenvironment that recapitulates human GBM. Surprisingly, the efficacy of CPI (anti-PD-1 + anti-CTLA-4) treatment in SB28 was site-dependent. Subcutaneous SB28 flank tumors were eradicated by CPI in all mice, whereas

intracranial tumors were refractory. CyTOF analysis revealed that MHC-II+ intratumoral macrophages were twice as frequent in subcutaneous versus intracranial tumors. Similarly, dendritic cells were abundant in subcutaneous tumor-draining lymph nodes, but absent in intracranial tumor-draining (cervical) lymph nodes. Mice previously cured of subcutaneous tumors rejected intracranial tumors upon rechallenge. Taken together, these results suggest that subcutaneous tumors allow superior antigen presentation, resulting in priming and sustained immunological memory after CPI. We then investigated FLT3-ligand, an essential cytokine for dendritic cell maturation, as a therapy to improve antigen presentation in mice with intracranial tumors. We administered recombinant FLT3-ligand intraperitoneally for 10 days after engraftment of intracranial tumors in naive hosts. FLT3-ligand dramatically increased the frequency of dendritic cells and CD8+ T cells in spleens and cervical lymph nodes. Notably, FLT3-ligand monotherapy also caused durable rejection of intracranial tumors in 30% of mice. These results indicate that sufficient T cell priming can confer immunological control of GBM, even in a macrophage-rich, T cell-poor microenvironment.

IMMU-05. LATE EFFECTS OF INTRACRANIAL RADIATION INDUCES RESISTANCE TO IMMUNE CHECKPOINT BLOCKADE THERAPY THAT IS PARTIALLY REVERSIBLE WITH CSF-1R INHIBITION

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Primary and metastatic CNS malignancies remain incurable despite aggressive therapy with surgery and radiation. Immunotherapy has shown promise in many solid and hematologic malignancies, however, results have been disappointing in recurrent primary CNS tumors. Additionally, while upfront immune checkpoint blockade therapy (ICB) has shown equal efficacy intracranially and extracranially, these responses are abrogated in previously irradiated brain lesions. Together, we hypothesize that radiation modulates the brain microenvironment to permit tumor growth and suppress anti-tumor immunity. As such, we developed a mouse model whereby orthotopic transplantation of murine gliomas into previously irradiated normal brain results in a more aggressive tumor phenotype. Moreover, the ICB-sensitive glioma cell line, GL261, is resistant to ICB therapy when implanted into a previously irradiated brain microenvironment. Immunophenotyping revealed a decreased ratio of CD8:CD4 T cells within tumor-infiltrating lymphocytes isolated from previously irradiated mice though the relative frequency of neoantigen-specific CD8 T cells was slightly increased and no difference in PD-1 expression was observed. Alternatively, the frequency of microglia and tumor-infiltrating $CD11b^{+}$ $Gr\text{-}1^{+}$ myeloid-derived suppressor cells (MDSC) was increased following irradiation suggesting a potential role for these myeloid cells in the immunosuppressive effects noted. Consistently, the administration of a CSF-1R inhibitor, which has been shown to reduce the number of microglia and block MDSCs, partially resensitizes GL261 cells to ICB therapy. In summary, we have developed a model that recapitulates the late effects of radiation on immunotherapy-resistance in CNS tumors. Preliminary results suggest that these late radiation effects are mediated through an increased myeloid population, and that inhibition of these cell subsets via the CSF-1R pathway can partially restore efficacy of ICB therapy. Furthermore, this model may provide further insight into additional therapeutic strategies that can be used to overcome these mechanisms of resistance induced by radiation therapy in the CNS.

IMMU-06. ABSENCE OF THE AMINO ACID STRESS-SENSOR GCN2 REDUCES SUPPRESSIVE EFFECTS OF MDSCs IN GLIOMA

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Regulation of nutrient availability is a critical way by which tumors exert their immunosuppressive effects on the host. The glioblastoma microenvironment, in particular, is enriched in expression of indoleamine 2,3- dioxygenase 1 (IDO1), which depletes tryptophan from the microenvironment. Upon depletion of tryptophan, the amino acid stress kinase, general control non-derepressible 2 (GCN2), inhibits general protein synthesis, and upregulates a number of genes to promote the cellular survival. In this study, we explored the effects of a deficiency of the GCN2 pathway on infiltrating immune cells in the context of the glioma. Interestingly after GL-261 (Glioma mouse model cell line) injections in GCN2 knock out (KO), and wild type (WT) mice, GCN2 KO mice survived significantly (p_{value}:0.0066) longer compared to WT mice. *In-vivo* flow cytometric analyses of these mice, showed significant reduction in $CD45^{hi}CD11b^{+}$ (myeloid cell marker) and Ly6C (monocytic MDSC marker) population in GCN2 KO mice. To study the mechanisms responsible for this, we generated *in-vitro* bone-marrow-derived MDSCs from both WT and GCN2KO mice. Surprisingly, the *in-vitro* T-cell suppressive effects of GCN2 KO MDSCs were significantly attenuated, with a decrease in arginase-1 (Arg-1) expression as a potential mechanism for this observation. To validate this phenomena *in-vivo*, we injected GCN2 KO and WT mice with GL-261 tumor cells, and after 18 days post tumor injection, we isolated Gr-1 (pan-MDSC marker) positive cells from tumor bearing brains, and checked for Arg-1 level by RNA and protein. Our data, both by protein and RNA demonstrated significant reduction in Arg-1 levels. Importantly, *ex-vivo* isolated Gr-1 myeloid cells from tumor bearing brain of GCN2 KO and WT mice shows dramatically reduced suppressive capabilities. In conclusion, our data demonstrate the importance of GCN2 pathway on MDSC functionality, and provide insight on how amino acid sensing enzymes may regulate immunity in glioblastoma.

IMMU-07. IMMUNE PROFILES IN THE SAN FRANCISCO ADULT GLIOMA STUDY (AGS) USING IMMUNOMETHYLOMICS

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Glioma patients demonstrate abnormalities in peripheral blood leukocytes that have been associated with survival time. Here we assessed immune cell profiles in archival blood samples obtained 5–25 years ago using a novel epigenetic approach called immunomethylomics. We first validated the approach to measure the proportions of CD4 T cells, CD8 T cells, B cells, NK cells, neutrophils, and monocytes in archival blood using the new 850,000 feature EPIC Illumina methylation bead array. The immunomethylomic assay was shown to match the performance of multiparametric flow cytometry and thus is a highly accurate method for immune profiling. We next measured cell profiles in blood from 312 molecularly defined AGS subjects. In this cohort we enriched for patients with triple negative tumor subtypes (IDH wildtype, 1p19q negative, TERT non-mutant). Elevations in the neutrophil lymphocyte ratio (NLR) significantly increased with grade, whereas the proportions of T cells decreased with increasing grade. Using an established cut point of > 4.0 for the NLR revealed that this immune parameter was associated with shorter survival times in glioblastoma (median overall survival (mOS) 12.6 vs. 16.9 months) and non-glioblastoma (mOS 16.7 vs. 36 months) patients. Ongoing analyses of the cohort will be presented to evaluate the effects of age, gender, surgery, chemoradiation and tumor grade on the survival results. Because DNA based immune cell profiles do not require intact cells nor preserved proteins, they provide a powerful tool to glean potentially important immunologic information from historic stored samples that could not otherwise be utilized using current cytometry-based approaches.

IMMU-08. THE ROLE OF WNT SIGNALING ON T-CELL INFILTRATION IN GLIOBLASTOMA

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BACKGROUND: Glioblastomas (GBMs) are considered immunologically cold tumors, making it difficult to apply immunotherapeutic strategies. Oncogenic pathways intrinsic to the tumor or the microenvironment can influence T-cell infiltration. WNT/β catenin is one such pathway implicated in modulating T-cell infiltration in other solid malignancies. In this study, we examine the influence of WNT signaling on the infiltration of T-cells in GBM. METHODS: Using the TCGA dataset, we analyzed the mRNA expression of both β-catenin dependent (canonical) and independent (non-canonical) WNT ligands and CTNNB1 (β-catenin) in GBM. The expressions of these components were correlated with a T-cell signature (CD3e, CD3d, CD3g, CD4, CD8a, and CD8b) and survival. Additionally, multiple GSC cell lines, derived from human GBM samples, were profiled for different WNT ligands and cells with the highest and lowest expression were selected for further experiments. *In vitro,* we also utilized a T-cell migration assay to measure the effect of WNT ligands (WNT3a and WNT5a) on T-cell migration towards a GL261 monolayer. RESULTS: *In silico* analysis of the TCGA dataset revealed downregulation of most WNTs in GBM and

in 49 evaluated GSC cells lines. In cases with evidence of a T-cell signature, disease-free progression was 2-fold higher than those without evidence of a T-cell infiltration. Additionally, we observed a mutual exclusivity between the T-cell signature and β -catenin expression. WNT5a (non-canonical WNT ligand) had a significantly higher expression in the low-risk group (higher survival *p=1.42e-33) in GBM. Furthermore, *in vitro,* we observed a 2-fold increase in the migration ability of effector T-cells (CD4+ CD8+) towards the GL261 monolayer in presence of WNT5a. CONCLUSION: This data suggest that the WNT/β-catenin dependent pathway negatively regulates T-cell infiltration whereas the β-catenin independent pathway may have a promoting role.

IMMU-09. HETEROGENEOUS INTRA-TUMORAL ANTIGEN EXPRESSION IN RELATION TO IMMUNOTHERAPY OF HIGH GRADE GLIOMA

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Glioblastoma (GBM) remains an almost universally fatal brain tumor despite numerous on-going efforts to devise novel and efficacious therapies. We are exploring one approach, immunotherapy with genetically modified T cells expressing chimeric antigen receptors (CARs) targeting specific tumor antigens. While adoptive CAR T cell immunotherapy has shown promising efficacy in pre-clinical and clinical studies, tumor relapses nevertheless occur, a reflection of our incomplete understanding of target antigen expression in GBM. We have previously suggested from analysis of inter-tumoral heterogeneity in primary tissues from patients with GBM that, in principle, a multiple antigen approach targeting IL13Rα2, HER2 and EGFR could effectively target approximately 93% of patient tumors. Our most recent observations, working with this same cohort of patient tumor samples, indicate that individual GBM lesions are composed of numerous subpopulations of tumor cells with differing target antigen expression patterns. This high degree of intra-tumoral heterogeneity, evident in the spatial extent ("granularity") of target antigen expression patterns and their relations to tumor architecture, may complicate single- and multiple-target immunotherapies by contributing to antigen escape and tumor recurrence.

IMMU-10. RADIOTHERAPY AND PD-1 BLOCKADE INCREASES TRYPTOPHAN METABOLISM IN BRAIN TUMOR-DRAINING SECONDARY LYMPHOID ORGANS

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INTRODUCTION: Glioblastoma (GBM) is an aggressive, incurable, primary brain tumor with a median survival of 15–20 months. High intratumoral expression of indoleamine 2,3-dioxygenase 1 (IDO1), an immunosuppressive enzyme that metabolizes tryptophan (Trp) into kynurenine (Kyn), is one factor that contributes to immunosuppression in GBM. Unexpectedly, we discovered that IDO1 enzyme activity becomes targetable in non-tumor cells after treatment with brain tumor radiation (RT) and PD-1 mAb (Ladomersky *et al*., 2018; CCR). The premise for this investigation is to evaluate IDO1 enzyme activity before and after radiation and PD-1 blockade, for the identification of non-brain tumor tissues that are active for immunosuppressive tryptophan metabolism. METHODS: Wild-type (WT) and IDO1 knockout mice (IDO1KO) were intracranially-injected (ic.) with 2×10^5syngeneic GL261. At two weeks post-ic., mice were treated with RT (n=10/group) or PD-1 mAb. Mice were euthanized at day one (n=5/group) or day seven, following treatment (n=5/group). IDO1 metabolism was evaluated by HPLC for Trp and Kyn levels in the brain tumor, contralateral non-tumor brain, cervical lymph nodes and spleen. RESULTS: Radiation had an early effect at increasing the Kyn/Trp ratio, a proxy for IDO1 enzyme activity, in normal brain and draining lymph nodes ($P<0.01$). Interestingly, PD-1 blockade also led to an early increase in the Kyn/Trp ratio of lymph nodes ($P<0.01$), but not in normal brain. In contrast, spleen showed a late increase in the Kyn/Trp ratio (P<0.01). Strikingly, the majority of Kyn/Trp ratio changes were observed both in WT and IDO1KO mice. CONCLUSION: Our data suggest a systemic and tissue-selective change in Trp metabolism after radiation and PD-1 blockade. Unexpectedly, many of these changes were not specific to IDO1, suggesting a therapeutic inducibility of other immunosuppressive mediators, such as tryptophan dioxygenase (TDO). These data suggest that, effectively inhibiting tryptophan metabolism in subjects with brain tumors will likely require dual IDO1/TDO inhibition.

IMMU-11. IDENTIFICATION OF GLIOMA-ASSOCIATED REGULATORY B CELLS AND EFFECTS OF RITUXIMAB IMMUNOTHERAPY

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In the context of glioblastoma multiforme (GBM), immunotherapy remains a promising approach. However, the potent immunosuppression induced by GBM is one of the primary obstacles to finding effective immunotherapies. This immunosuppression is associated with high levels of immunosuppressive cytokines and a significant accumulation of both regulatory T cells (CD4+CD25+FoxP3+, Tregs) and myeloid-derived suppressor cells (MDSCs) within the tumor microenvironment. Other actors of the immune system may also play a role in glioma immunobiology, such as B cells, known to infiltrate gliomas. From a total of 60 GBM samples, we observed that 40% of GBM samples scored positive for $CD20^+$B-cell infiltration, presenting a discrete perivascular distribution. The current fundamental work aims to elucidate the phenotype and function of glioma-infiltrating B cells in two different glioma experimental model, GL261 and CT2A. Our results showed that glioma-associated B cells are a unique activated B-cell subset that produces immunoregulatory cytokines IL-10, TGFb and IL35; and inhibitory ligands PD-L1 and the polivirus receptor CD155. Glioma-associated Bregs are activated by MDSC and interact with activated CD8 T cells and strongly suppress their expansion and cytotoxic function, suggestive of their pro-tumorigenic function. In accordance, B-cell depletion therapy (anti-CD20; Rituximab) improved significantly animal survival. This work highlights the potential involvement of B cells in glioblastoma physiopathology and provides crucial information about the mechanisms by which gliomas is able to modulate B-cell immunity.

IMMU-12. TGFβ ACTIVATION BY RADIATION OPPOSES IMMUNE REJECTION OF INTRACRANIAL GL261

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Enabling anti-tumor immunity in brain is a challenge due to its unique microenvironment that includes tissue-specific extracellular matrix, immune cells and vasculature, and because many glioblastoma patients require rapid treatment, usually surgery followed by radiation therapy, that may oppose immunotherapy. Here we hypothesize that transforming growth factor β (TGFβ) is at the root of the profoundly immunosuppressive tumor microenvironment, and is perpetuated by standard of care, radiation therapy. We first localized TGFβ activation in situ using GC1008, a humanized pan-isoform TGFβ neutralizing antibody, radiolabeled with ^{89}Zr for PET-CT imaging. The antibody localized to a murine intracranial tumor compared to the injury-control brain injected with PBS. Paired comparisons of dual flank tumors in which one was irradiated (15 Gy) showed that radiation significantly increased ^{89}Zr-GC1008 uptake (p0.0002). This was confirmed by immunostaining with an antibody that detects active TGFβ and nuclear pSMAD, indicative of signaling. Administration of TGFβ pan-isoform neutralizing antibody, 1D11 (25 mg/kg), to mice bearing irradiated intracranial tumors reduced immunostaining for active TGFβ and p-SMAD and blocked induction of a critical TGFβ target, tenascin-C, compared to treatment with isotype control antibody. These data support radiation-induction of TGFβ activation. Mice bearing i.c. GL261 (n=10–12) treated with 1D11 compared to isotype IgG had similar (17d vs 16d) median survival, which was doubled by tumor irradiation (10 Gy). Combined treatment with 1D11 and radiation led to durable control (Kaplan-Meier, p>0.0009), in which mice that showed complete regression by bioluminescence imaging for >45 days rejected a flank GL261 re-challenge. TGFβ inhibition with tumors treated with 5 daily 6 Gy fractions also eradicated most intracranial GL261. The undetectable brain bioluminescence and successful re-challenge suggest that TGFβ inhibition in the context of radiation can release the immunosuppressive microenvironment, elicit anti-tumor immunity and enable immune memory.

IMMU-13. EFFECT OF COMBINED ANTI-PD-1 AND TEMOZOLOMIDE THERAPY IN GLIOBLASTOMA

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Although programmed death-1 (PD-1) blockade is effective in treating several types of cancer, the efficacy of this agent in glioblastoma (GBM) is

largely unknown. We evaluated therapeutic effects of anti-PD-1, temozolomide (TMZ), and their combination in an orthotopic murine GBM model. The phenotype, number, and composition of lymphocytes were evaluated using flow cytometry. Transcriptional profiles of tumor tissues were analyzed using microarrays. Generation of antitumor immunological memory was investigated upon rechallenge. Combined treatment with anti-PD-1 and TMZ yielded synergistic antitumor efficacy in the presence of donor-originated PD-1^{+}CD8^{+} T cells *in vitro*, necessitating *in vivo* validation. Whereas TMZ did not rescue GBM-implanted mice, anti-PD-1 completely eradicated GBM in 44.4% of mice, and combination of anti-PD-1 and TMZ in all mice. Anti-PD-1 significantly increased the number of tumor-infiltrating lymhpocytes (TILs), and reduced frequencies of exhausted T cells and regulatory T cells. However, combining TMZ with anti-PD-1 significantly decreased the number of TILs, which was also observed with TMZ treatment alone. A transcriptome analysis of tumor tissues revealed that anti-PD-1 monotherapy perturbed immune-related genes, distinctly with combined therapy. Upon rechallenge, tumor growth was not observed in mice cured by anti-PD-1 monotherapy, whereas tumors regrew in the combination group. Furthermore, an analysis of peripheral blood revealed that antitumor memory T cells were generated in mice cured by anti-PD-1 monotherapy, not in the combination group. PD-1 blockade induces long-term therapeutic response, and combination with TMZ further enhances antitumor efficacy. However, immunological memory is provoked by anti-PD-1 monotherapy, not by combined therapy.

IMMU-14. THERAPEUTIC MODULATION OF THE PHAGOCYTIC AXIS SPARKS ANTI-TUMOR CD8 T CELL RESPONSES IN GLIOBLASTOMA

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As the most common primary brain tumor in adults, Glioblastoma (GBM) remains a major unmet medical need. With current treatment strategies, the median survival remains approximately 15 months, and recurrence occurs in nearly all cases. In this study we examine a novel role for the DNA-methylating agent temozolomide (TMZ) as an activator of innate immunogenicity in GBM. TMZ-mediated DNA damage promotes calreticulin (CRT) translocation to the plasma membrane of cancer cells where it functions as a driver of phagocytosis. Ancillary blockade of anti-phagocytic signaling through Cluster of Differentiation 47 (CD47) further enhances tumor cell uptake by bone-marrow derived macrophages (BMDM). Together these agents promote maturation of BMDM into antigen presenting cells (APCs), capable of initiating effector T cell responses in vitro. We recapitulate these findings in immune-competent preclinical models of GBM, where combination therapy significantly prolongs survival in a cytotoxic CD8+ T cell dependent manner. The results of this study indicate that phagocytic axis modulation is a novel strategy to reprogram the innate immune microenvironment, shifting the dynamic towards an 'inflamed' tumor phenotype. This novel approach to immunotherapy in GBM is highly translational and warrants further investigation in the clinical setting.

IMMU-15. ENGINEERED-DRUG RESISTANT GAMMA-DELTA (γδ) T CELLS COMBINED WITH IMMUNE CHECKPOINT BLOCKADE AUGMENTED KILLING OF CANCER CELLS

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We have previously shown the potential for dramatic improvement in overall survival in immunodeficient mice bearing patient-derived xenograft (PDX) models of primary and temozolomide (TMZ)-resistant GBM when treated using a combination of intracranial therapy with *ex-vivo* expanded/activated MGMT-engineered γδT cells and simultaneous systemic TMZ. We have termed this approach Drug-Resistant Immunotherapy (DRI). TMZ upregulates γδT cell stress-antigen targets (NKG2DL) on primary and TMZ-resistant tumors making them more visible to effector γδT cells. In the present study, we sought to determine whether checkpoint inhibition would potentiate the effect of DRI in our PDX model system for glioma. Expanded/activated δT cells were evaluated for lysis of target cells K562 and disaggregated PDXT cells in the presence or absence of checkpoint inhibitors of PD-1, CTLA-4 and PD-L1 using a flow cytometric based killing assay. Cytotoxicity was increased by anti-CTLA-4 and anti-PD-L1 against JX22T PDXT, although a much more noticeable effect of blockade with anti-PD-1, anti-CTLA-4 and anti-PD-L1 was seen against K562. Anti-PD-1 combined with anti-CTLA-4 also showed a synergistic effect on JX22T and K562. Concurrently, we also demonstrated that although TMZ did not influence the already low expression of PD-L1 in disaggregated PDXT lines exposed to 200μM TMZ, immunohistochemical analysis of tumors from mice injected with 60mg/kg TMZ and examined 4h later showed upregulation of NKG2DL accompanied by modest upregulation of PD-L1. At the time of this writing, mice bearing JX12P and JX12T were still under examination. These early studies show that checkpoint inhibition can potentiate the cytotoxic activity of expanded/activated γδT cells. Also, TMZ may increase both the expression of NKG2DL and PD-L1 in some tumors. Taken together, our findings justify further exploration of combination DRI and checkpoint inhibition either by systemic administration, as part of the therapeutic graft, or both.

IMMU-16. GUADECITABINE (SGI-110) ENHANCES MHC class I AND TUMOR ANTIGEN EXPRESSION ON MURINE C57BL/6-SYNGENEIC GLIOMA AND DIPG MODELS

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Diffuse intrinsic pontine glioma (DIPG) is one of the most lethal pediatric brain tumors, with a median survival time of less than one year. As DIPG tumors are insensitive to chemotherapy and surgically inaccessible, there is an urgent need for the development of novel therapeutic approaches. Our group and others have been evaluating peptide vaccine immunotherapies that target glioma-associated antigens (GAAs). Enhancing the expression of these immunogenic GAAs and MHC I on tumor cells may promote immune-mediated tumor recognition and killing following peptide vaccine immunotherapy. Accordingly, DNA methyltransferase (DNMT) inhibitors have been shown to augment the expression of MHC, tumor antigens, and other immunosensitizing molecules. Guadecitabine (SGI-110), a next-generation DNMT inhibitor prodrug, has been developed to prolong tumor cell exposure to its active metabolite, decitabine. In the current study, we evaluated whether SGI-110 can immunosensitize murine glioma cells to peptide vaccine immunotherapy by enhancing their surface expression of MHC I and a GAA, EphA2. We developed a novel C57BL/6-syngeneic DIPG model by culturing cells from a Sleeping Beauty *de novo* glioma-induced in neonatal mice using a K27M-mutated histone 3.3 plasmid and other oncogenic plasmids (SB-DIPG-11). Flow cytometry analysis showed that SB-DIPG-11 cells express both murine MHC I (H-2Kb/H-2Db) and EphA2 on their surface. *In vitro,* treatment of SB-DIPG-11 and C57BL/6-syngeneic GL261 cells with SGI-110 resulted in a dose-dependent increase of MHC I and EphA2 surface expression. Based on these data, we are evaluating whether SGI-110 can improve the efficacy of peptide vaccine immunotherapy targeting EphA2 *in vivo* using our new DIPG mouse model. Our current data demonstrate that SGI-110 may promote antigen and MHC I expression to improve peptide vaccine immunotherapy for children with DIPG.

IMMU-17. PEPTIDE VACCINE IMMUNOTHERAPY BIOMARKERS AND RESPONSE PATTERNS IN PEDIATRIC GLIOMAS

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Low-grade gliomas (LGGs) are the most common brain tumor affecting children. We recently reported an early phase clinical trial of a peptide-based vaccine, which elicited consistent antigen-specific T cell responses in pediatric LGG patients. Additionally, we observed radiologic responses of stable disease (SD), partial response (PR), and near-complete/complete response (CR) following therapy. To identify biomarkers of clinical response in peripheral blood, we performed RNA sequencing on PBMC samples collected at multiple time points. Patients who showed CR demonstrated elevated levels of T cell activation markers, accompanied by a cytotoxic T cell response shortly after treatment initiation. At week 34, patients with CR demonstrated both IFN signaling and Poly-IC:LC adjuvant response patterns. Patients with PR demonstrated a unique, late monocyte response signature. Interestingly, *HLA-V* expression, before or during therapy, and an early monocytic hematopoietic response were strongly associated with SD. Low *IDO1* and *PD-L1* expression before treatment and early elevated levels of T cell activation markers were associated with prolonged progression-free survival. Furthermore, we identified that genes associated with dendritic cell activation of T-cells correlated with IFNγ ELISPOT counts. Currently we are validating the observed response patterns and biomarkers in high-grade glioma patients treated with peptide vaccine immunotherapy. Overall, our

data support the presence of unique peripheral immune patterns in LGG patients associated with different radiographic responses to our peptide vaccine immunotherapy. Future clinical trials, including our ongoing phase II LGG vaccine immunotherapy, should monitor these response patterns.

IMMU-18. TARGETING THE PD1 AND TIGIT CHECKPOINT PATHWAYS FOR ADULT AND PEDIATRIC GLIOMAS

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High-grade gliomas (HGGs) are the most common and deadliest of malignant primary brain tumors. Additionally, brain and central nervous system tumors are the leading cause of cancer-related morbidity and mortality in infants and children. Tumors, including HGGs, co-opt immune checkpoint molecules to evade T-cell killing, and targeting these pathways has led to cures in some patients with cancers. Immunotherapy trials, including the use of checkpoint blockade therapies, have been conducted with limited success in brain tumor patients. Our analysis of data from The Cancer Genome Atlas identified that of over 30 assessed checkpoint molecules, only expression of the genes coding for PD1 and TIGIT, within the tumor, were associated with shorter overall survival and progression-free survival times. Furthermore, using published pediatric glioma resources, we found that TIGIT expression is elevated in pediatric HGGs compared with lower-grade pediatric gliomas, and TIGIT expression was inversely associated with overall survival in children with HGGs. These data led us to hypothesize that glioma-associated T-cells express PD-1 and TIGIT, which can be targeted to reduce glioma growth. To test this, we assessed whether mono/combination therapies with anti-PD-1 and anti-TIGIT could improve survival and immune function in a syngeneic orthotopic GL261 glioma mouse model. Although anti-TIGIT therapy alone failed to prolong survival, mice treated with both anti-PD1 and anti-TIGIT demonstrated improved survival compared with mice receiving anti-PD1 alone. Immunologic analysis of tumor-infiltrating cells in our mouse model revealed high expression levels of PVR, the TIGIT ligand, on cells resembling myeloid-derived suppressor cells (MDSCs), within the tumor. Additionally, TIGIT treatment led to the reduction of a subset of T-regulatory cells and cells resembling MDSCs. Overall, our data suggest that anti-TIGIT may reduce immunosuppressive cells at the tumor site and improve the efficacy of anti-PD1 trials in adults and children with HGGs.

IMMU-19. PD-1 BLOCKADE ACTIVATES CD4 T CELLS AND THE INNATE IMMUNE RESPONSE FOR GLIOBLASTOMA ERADICATION

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Blockade of immune cell co-inhibitory receptor PD-1 using monoclonal antibodies (mAbs) allows the development of anti-tumor immunity in various solid tumors and lymphoid malignancies. We recently demonstrated that PD-1 blockade elicits an anti-tumor immune response resulting in tumor rejection and long-term survival in approximately 50% of mice with intracranial GL-261 glioblastoma, despite the absence of accumulating CD8+ cytotoxic T cells in the tumor or draining lymph nodes. In this investigation, we provide evidence for the role of conventional CD4+ T cells and the innate immune response in PD-1 mediated anti-glioma immunity in this model. In response to anti-PD-1 monotherapy, intratumoral CD4+ T cells, but not CD8+ T cells, expressed significantly elevated levels of IFN-γ and TNF-α pro-inflammatory cytokines and the cytotoxic enzyme, granzyme B. Tbet, GATA3, and EOMES, transcription factors required for T cell proliferation, activation, and effector function, were also up-regulated in CD4+, but not CD8+ T cells in the brains of mice treated with PD-1 mAbs when compared to controls. We demonstrated that depletion of CD4+ or CD8+ T cells, but not NK, was sufficient to completely ablate anti-PD-1-mediated tumor eradication and long-term survival. CD4+ T cell activation was accompanied by the classical activation and M1 polarization of resident microglia and tumor-infiltrating macrophages. Together, these studies demonstrate for the first time a role for CD4+ T cells and the innate immune response in the eradication of glioblastoma by PD-1 blockade.

IMMU-20. SINGLE CELL CYTOMICS OF PERIPHERAL BLOOD MONONUCLEAR CELLS REVEALS NEW AVENUES FOR GLIOMA IMMUNOTHERAPY

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Glioblastoma is the most common primary brain tumor with a dismal prognosis and a lack of effective treatment options after relapse. Despite striking successes that were achieved in the last decade in other types of cancer, evidence of sustainable responses to T cell directed immunotherapy in glioblastoma remains limited. This might be explained by an emerging hypothesis that glioblastomas are cold tumours with poor lymphocyte infiltration. Therefore, some recent efforts have been focused on increasing cytotoxic lymphocyte infiltration. Here we present our workflow for subpopulation frequency, marker expression, and correlation network (INFERENCE) analysis, in this study applied to screen for possible new targets to enhance anti-glioblastoma immune responses. Using high-dimensional, single-cell mass cytometry (CyTOF) we profiled peripheral blood mononuclear cells derived from 160 patients with glioblastoma, lower grade glioma, or metastasis, meningioma and epilepsy as controls. Based on a CyTOF panel composed mainly of lymphocyte markers and check-point inhibition markers we could effectively dissect blood leukocytes into 100 phenotypically separate subsets of CD4 T cells (35 subsets), CD8 T cells (17 subsets), monocytes (12 subsets), NK cells (14 subsets), and B cells (15 subsets), and map their frequencies, phenotypes and correlation statistics. We thereby created an in depth human atlas of peripheral blood mononuclear cells in patients with different types of intracranial tumors. Interestingly, a subset of B cells characterized by high expression of the Interleukin-2 receptor alpha chain (CD25, a typical marker of regulatory T cells) was significantly increased in glioblastoma patients. This immune suppressive B cell subset has previously been associated with enhancing the suppressive effect of regulatory T cells. Further research is warranted to investigate the role of this aberrant B cell subset in the context of glioblastoma and its potential as target for immunotherapy.

IMMU-21. MULTIDIMENSIONAL CHARACTERIZATION OF IMMUNE CELL POPULATIONS IN THE GLIOMA TUMOR MICROENVIRONMENT REVEALS A DOMINANT PROPORTION OF CELLS DERIVED FROM THE MYELO-MONOCYTIC LINEAGE

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INTRODUCTION: Immunotherapy represents an exciting new strategy to treat patients with malignant gliomas; however, we and others have previously shown that the presence of other immune cell populations in the tumor microenvironment can reduce the effectiveness of immunotherapy. To date, a comprehensive and quantitative examination of immune cell composition in the glioma microenvironment has not been performed. METHODS: We purified CD45+ cells from freshly digested tumor samples of 35 malignant glioma patients undergoing surgical resection at UCLA and performed CyTOF mass cytometry. We then analyzed the lineage and functional marker expression of these populations. Next, to pursue an unbiased analysis of the multi-dimensional data, we used a computational algorithm that clusters cells into phenotypically distinct populations in an unsupervised manner. RESULTS: CD11b+ myelo-monocytic cells (1542.13 ± 306.82 cells/mg of tumor; 51.33 ± 6.43% of all cells) represented the dominant population and were more abundant than CD3+ T lymphocytes (233.65 ± 59.53 cell/mg of tumor; 8.41 ± 1.47% of all cells; $p < 0.001$). Meanwhile, our unsupervised clustering algorithm generated 12 distinct phenotypic clusters. Amongst the immune cells, 76.81% were grouped into 6 clusters that derived from populations of a monocytic lineage including a classical monocytic population, an inflammatory monocyte population, and a differentiated M2 macrophage population. 12.37% of the tumor-infiltrating immune cells grouped into 2 clusters of a NK lineage, 9.06% grouped into 2 clusters of a T cell lineage, 0.3% grouped into 1 cluster of a B cell lineage, and 1.46% cells in 1 cluster of an unable to be classified cell lineage. CONCLUSIONS: Cells of monocytic origin represented the dominant fraction of cells within the tumor-infiltrating immune cell population. Understanding the immune cell composition in the tumor microenvironment may help improve current and future immunotherapies against gliomas.

IMMU-22. PHARMACOLOGICALLY TARGETING EXTRACELLULAR VESICLE-IMMUNE CELL INTERACTIONS IN GLIOBLASTOMA

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Glioblastoma (GBM) is the most aggressive type of primary brain cancer and carries a dire prognosis. Increasing evidence points to extracellular

vesicles (EVs) as a means by which GBM tumor cells modulate the tumor microenvironment, leading to immunosuppression and disease progression. Several pharmacologic agents may inhibit EV release (fasudil, a ROCK1 inhibitor; GW4869, a Rab27B inhibitor) or block EV interactions with target cells via heparin sulfate proteoglycan (heparin, an HSPG competitive inhibitor). We sought to characterize the effects of these drugs on EVs release from GBM cells and binding to target cells. EVs released by the human GBM cell line BT116 were quantified by nanoparticle tracking in with the presence or absence of the putative release inhibitors fasudil and GW489. Compared to untreated cells, pharmacologically treated BT116 cells demonstrated modest reductions in EV release with 50uM fasudil (17%) and 10uM GW4869 (31%). We have previously demonstrated that BT116 EVs can induce immunosuppressive monocyte development including myeloid-derived suppressor cells (MDSCs) and non-classical monocytes (NCMs). Under confocal microscopy, monocytes conditioned with GBM-derived EVs demonstrated markedly reduced EV uptake in the presence of heparin than untreated cells in a dose-dependent manner (1uM-50uM heparin). In the presence of heparin, BT116 EVs induced reduced NCM formation when cocultured with normal donor-derived monocytes, but did not appear to affect MDSC differentiation. Taken together, these findings point to druggable targets in the interaction between glioblastoma-derived EVs and host immune cells. This may prove important in treating GBM-mediated immunosuppression and optimizing GBM immunotherapy.

IMMU-23. ANTIGEN-SPECIFIC EFFECTOR MEMORY CD4+ T CELLS AND CCL3 POTENTIATE DENDRITIC CELL VACCINES AND ANTITUMOR IMMUNITY

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INTRODUCTION: Efficacy of dendritic cell (DC) vaccines for glioblastoma (GBM) has been limited by suboptimal migration to draining lymph nodes. Protein antigens induce robust T cell responses that can potentiate innate antigen presenting cells such as DCs. Our previous work in patients with GBM and corroborating mouse models receiving tumor antigen-specific DCs revealed that migration and antitumor responses were significantly enhanced by CD4+ T cell memory responses to a different protein antigen, tetanus-diphtheria (Td) toxoid. Furthermore, vaccine responses were dependent on the chemokine CCL3. Here, we investigated how memory CD4+ T cells interact with cognate antigens Td and the *Mycobacterium tuberculosis* antigen 85B (Ag85B) to enhance responses to ovalbumin (OVA)-expressing DCs and tumors. METHODS: C57BL/6 and CD4 + 85B TCR transgenic mice were used to study the effects of Td and Ag85B-specific responses on OVA-DC migration and efficacy. RESULTS: One day following Td and Ag85B preconditioning, there is an influx of CD4+ T cells in the skin site (p=0.002), and this is dependent on the protein antigen recall response (p=0.005). Both Td and 85B preconditioning led to locally induced CCL3 and increased migration of OVA-DCs (Td, p=0.002; Ag85B, p=0.001). Depletion of CD4+ T cells resulted in a loss of induced CCL3 as well as abrogation of increased DC migration (p=0.005). Mice with established B16/F10-OVA tumors receiving Td preconditioning demonstrated suppressed tumor growth compared to controls, which was abrogated in *Ccl3-/-* hosts. However, adoptive transfer of Td memory CD4+ T cells and exogenous CCL3 rescued the effect of suppressed growth (Day 21 mean tumor volumes, p=0.017). CONCLUSIONS: The induction of CD4+ memory responses to protein antigens can sufficiently facilitate the increased migration of tumor antigen-specific DCs and promote antitumor immunity. This axis of the adaptive immune response relies on host CCL3, which can serve as a potent immunotherapy adjuvant in future studies.

IMMU-24. THE IMPACT OF BRAIN TUMORS ON HEMATOPOIETIC STEM CELL-DERIVED T CELLS

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INTRODUCTION: The T cell repertoire of brain tumor bearing hosts has been previously described to be skewed to have increased regulatory phenotype relative to healthy hosts (Woroniecka *et al* 2018). We have found that hematopoietic stem cells (HSCs) isolated from tumor bearing mice are inefficient at engraftment and reconstitution of the hematopoietic compartment, giving rise to less live cells than HSCs derived from healthy controls. We found that CD4+ splenocytes and CD8+ bone marrow-derived cells and splenocytes that arise from HSCs of tumor bearing hosts are polarized towards a terminal memory phenotype relative to HSCs derived from healthy hosts. We believe that this dysregulation in T cell reconstitution is a major player in mounting immune responses against CNS malignancies. METHODS: Lineage negative HSCs were isolated from GFP transgenic healthy donors or DsRed transgenic tumor-bearing mice and adoptively transferred into C57BL/6 recipient cohort of lethally irradiated mice. HSCs are derived from DsRed or GFP transgenic mice to allow for tracking of HSC-derived populations. One month after transfer, mice are sacrificed and spleen, bone marrow, and blood are harvested and stained for flow cytometry. RESULTS & CONCLUSIONS: T cells derived from TB HSCs have a distinct phenotype compared to T cells from healthy HSCs, demonstrating intracranial brain tumors likely have an impact on HSC differentiation outcomes.

IMMU-25. PROGRAMMED CELL DEATH-LIGAND 1 (PD-L1) IS NOT EXPRESSED IN DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) TUMOR CELLS

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BACKGROUND: Diffuse intrinsic pontine glioma (DIPG) is a pediatric high grade, infiltrative tumor that arises in the pons. It is the leading cause of brain tumor related death in children. Despite increased understanding of the genetics and epigenetics of DIPG, development of in vivo research models, and several clinical trials, standard of care and survival has not improved. One avenue that has yet to be explored in DIPG is the use of immune checkpoint inhibitors such as the anti-PD-1 and PD-L1 monoclonal antibodies, Nivolumab and Pembrolizumab, respectively. PD-1/PD-L1 checkpoint inhibition is currently being used to treat certain solid tumors, including melanoma and non-small cell lung cancer. In addition, two pediatric glioblastoma multiforme cases secondary to bi-allelic mismatch repair deficiency responded to treatment with Nivolumab. Evidence of immune infiltration and PD-1/PD-L1 expression was seen in these two tumors. Similarly, there may be a subset of DIPG that may respond to inhibiting the PD-1/PD-L1 axis. METHODS: To test this hypothesis, immunohistochemistry was performed for PD-L1 (SP142 clone), CD4 and CD8 in DIPG tumor samples using formalin-fixed paraffin-embedded tissues from autopsy, and appropriate positive and negative controls. PD-L1 (n=31) was evaluated as percentage of positive tumor cells. CD4 and CD8 (n=20) expression was assessed from 0 to 3+ based on average number of positive lymphocytes in four high power fields. RESULTS: Membranous PD-L1 expression was negative in each of the 31 cases characterized. There was low expression of CD4 (1+) in 11 out of 20 cases and CD8 was moderately expressed in 19 cases (1–2+). CONCLUSIONS: Targeting the PD-1/PD-L1 axis may have limited efficacy against DIPG. However, since tumor infiltrating lymphocytes are present, alternate immune evasion mechanisms, such as IDO and CTLA-4, should be evaluated in order to further characterize the immune microenvironment and assess the potential of immunotherapy against DIPG.

IMMU-26. VISUALIZING TUMOR CELL - LYMPHOCYTE INTERACTIONS IN THE BRAIN METASTATIC CASCADE USING IN VIVO TWO PHOTON MICROSCOPY

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Despite high frequency of brain metastases (BM) in patients with advanced tumor stages and immunotherapy becoming the treatment of choice in these cases, the mechanisms of the adaptive immune response in BM is poorly understood. Current knowledge on the immunological tumor microenvironment is gained from *in vitro*- and *ex vivo* experiments. However, these methods fail to recapitulate the dynamic influx of lymphocytes and their interaction with tumor cells in the complex tumor microenvironment. A better understanding of lymphocyte recruitment, trafficking and activation *in vivo* is urgently needed to improve immunotherapeutic approaches. To characterize the adaptive immune response in BM at single cell resolution, we developed a unique model allowing us to monitor these processes *in vivo* using repetitive two photon intravital microscopy. Recipient mice with a chronic cranial window were introduced with fluorescent glycoprotein-100 (gp100) positive syngeneic melanoma cells via heart injection or stereotactic injection into the cortex. Next, these mice received adoptive cell transfer of tumor-specific, fluorescently labeled CD8+ T cells from pmel-1 donor mice. It was possible to establish the first long-term imaging method to study dynamic lymphocyte- tumor cell interactions in the event of BM *in vivo* on a single cell level. CD8+ T Cells could be recognized not only by its fluorescence signal but also due to their size and typical lymphocytic movement. We observed tumor-specific T cells to cross the brain blood barrier within a couple of hours and reside at the site of tumor for several days. Notably,

transferred T cells were found in the tumor margin and inside the tumor but not in healthy brain parenchyma. Taken together, this preclinical model provides insights into the underlying mechanisms driving the immuno-response *in vivo* at high resolution. Importantly, it enables to further investigate and improve immunotherapeutic approaches with respect to prevention and therapy of BM.

IMMU-27. PREVENTING T-CELL S1P1 INTERNALIZATION OBVIATES BONE MARROW T CELL SEQUESTRATION AND IMPROVES IMMUNOTHERAPEUTIC EFFICACY IN GBM

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INTRO: Glioblastoma (GBM) is among the most immunosuppressive of solid tumors, eliciting profound lymphopenia and T-cell dysfunction that limit immunotherapeutic success. This lymphopenia, as well as marked lymphoid organ contraction, is recapitulated in murine models of GBM. The bone marrow is the lone lymphoid organ not subject to T-cell loss, instead sequestering large numbers of naïve T-cells. We have previously shown that loss of the sphingosine-1-phosphate receptor type 1 (S1P1) from the T-cell surface plays a role in mediating this sequestration in both mice and patients with GBM. Here we show that stabilization of the receptor on T-cells reverses their sequestration and licenses T-cell activating therapies that were previously ineffective. METHODS: Blood, bone marrow, and tumors were collected from mice bearing CT2A gliomas and analyzed by flow cytometry. T-cell S1P1 levels were assessed, as were T-cell counts in each compartment. S1P1 receptor stabilization was achieved with a knock-in model (S1P1KI) in which receptor internalization is inhibited. Adoptive transfer experiments utilized tail vein injections of $1x10^4$ splenocytes. For treatment experiments, survival was assessed by Kaplan-Meier estimator. RESULTS: Sequestered T-cells in tumor-bearing mice show decreased surface S1P1 levels. Genetic stabilization of the S1P1 receptor through obviated internalization abrogates T-cell sequestration. Adoptively transferred T-cells with stabilized receptor resist sequestration when transferred into tumor bearing mice, and S1P1KI mice do not show T-cell sequestration following tumor implantation. S1P1KI mice also show increased numbers of activated tumor-infiltrating T-cells. While S1P1 stabilization alone does not extend survival, S1P1KI mice display enhanced survival when treated with 4-1BB agonist +/- anti-PD-1. CONCLUSION: S1P1-mediated bone marrow T-cell sequestration is a novel mode of cancer-induced T-cell dysfunction in GBM. Preventing receptor internalization abrogates T-cell sequestration and licenses T-cell activating therapies. Pharmacologic strategies to reverse sequestration and restore circulating T-cell counts are anticipated to improve immunotherapeutic efficacy for GBM.

IMMU-28. HIGH-DIMENSIONAL SINGLE CELL CHARACTERIZATION OF THE SYSTEMIC INFLUENCE OF NEOADJUVANT PD-1 BLOCKADE IN PATIENTS WITH RECURRENT GLIOBLASTOMA

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INTRODUCTION: PD-1 blockade has demonstrated efficacy in numerous cancer types; however, responses vary widely. Consequently, numerous studies have sought to identify reliable biomarkers that predict response to PD-1 inhibition. Documented responses to PD-1 blockade in GBM are limited to patients with hypermutated phenotypes, which are rare in this disease. METHODS: We opened a multi-institutional pilot study of pembrolizumab in patients with recurrent, surgically resectable GBM, randomizing patients 1:1 to receive neoadjuvant plus adjuvant pembrolizumab or adjuvant-only therapy. We hypothesized that neoadjuvant PD-1 blockade would lead to statistically significant differences in peripheral immune cell populations, using time-of-flight mass cytometry (CyTOF) to achieve our aims. RESULTS: Patients who received neoadjuvant pembrolizumab demonstrated decreases in a $CD33^+CD11b^+CD11c^+CD14^+CD16^+$ intermediate monocyte population between baseline and after surgery (adjusted P value = 0.027), whereas no changes were seen in the adjuvant-only group. When evaluating the median expression of markers of antigen experience and activation on various cell populations, we noted increases in CD127 and CTLA4 (P = 0.016 and 0.006, respectively) as well as decreases in PD-1 and CD25 (P = 0.016 and 0.027, respectively) expression on $CD4^+$ T-cells in the neoadjuvant group before and after the first dose of pembrolizumab, but not the adjuvant-only cohort. On $CD8^+$ T-cells, there was a significant decrease in PD-1 expression in the neoadjuvant group only (P = 0.004). Furthermore, in penalized regression survival analyses, a $CD4^+$ T-cell population has emerged as a potential predictor of clinical response. CONCLUSIONS: Leveraging time-of-flight mass cytometry, we have demonstrated that neoadjuvant PD-1 blockade elicits distinct changes in myeloid and lymphoid cell populations in patients with recurrent, surgically resectable glioblastoma. We hypothesize that this is reflective of tumor-specific T-cell activation in the presence of tumor antigens; these T-cells are subsequently expanded and activated in a tumor- and interferon-γ-driven process following surgical resection.

IMMU-29. COMBINATION PD-1 BLOCKADE AND IRRADIATION OF BRAIN METASTASIS INDUCES AN EFFECTIVE ABSCOPAL EFFECT IN MELANOMA

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Nearly half of melanoma patients develop brain metastases during the course of their disease. Despite advances in both localized radiation and systemic immunotherapy, brain metastases remain difficult to treat, with most patients surviving less than 5 months from the time of diagnosis. While both treatment regimens have individually shown considerable promise in treating metastatic melanoma, there is interest in combining these strategies to take advantage of potential synergy. In order to study the ability of local radiation and anti-PD-1 immunotherapy to induce beneficial anti-tumor immune responses against distant, unirradiated tumors, we used two mouse models of metastatic melanoma in the brain, representing BRAF mutant and non-mutant tumors. Combination treatments produced a stronger systemic anti-tumor immune response than either treatment alone. This resulted in reduced tumor growth and larger numbers of activated, cytotoxic $CD8^+$ T cells, even in the unirradiated tumor, indicative of an abscopal effect. The immune-mediated effects were present regardless of BRAF status. These data suggest that irradiation of brain metastases and anti-PD-1 immunotherapy together can induce abscopal anti-tumor responses that control both local and distant disease.

IMMU-30. HIGH-DIMENSIONAL PHENOTYPING OF IMMUNE SUBSETS AND CHECKPOINTS IN THE MOUSE GLIOBLASTOMA MICROENVIRONMENT

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Recent clinical trials in glioblastoma have yet to show any benefit of immune checkpoint inhibitors and are mostly driven by the fact that these inhibitors have efficacy in other cancer types. For immunotherapy to succeed in glioblastoma patients, we need to understand the immunological subsets and phenotypes involved to target and manipulate them. To this end, we set up different glioblastoma mouse models and used multiparameter flow cytometry and t-SNE unsupervised clustering of immune subsets including co-expression of immune checkpoints and their ligands. When comparing the brain tumor microenvironment with immune cells present in the contralateral hemisphere and the systemic compartment, we identified unique immune subsets. Different populations of infiltrating CD4 T cells were characterized by expression of TIGIT, PD-1 and HVEM. PD-1 expression was also significantly increased on infiltrating CD8 T cells. Furthermore, analysis of myeloid subsets showed massive infiltration of macrophages in the brain tumor microenvironment, which is also apparent in clinical glioblastoma samples. Co-expression of PD-L1, CD155 and BTLA was observed on both infiltrating macrophages and brain-resident microglia. Together, these results suggest a glioblastoma-induced 'tolerogenic' microenvironment and, while T cells are present, myeloid cells might prove to be a better target for combination immunotherapy.

IMMU-31. DYSFUNCTIONAL STING PATHWAY SIGNALING COMPROMISES INNATE IMMUNITY IN GLIOBLASTOMA

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BACKGROUND: Stimulator of interferon genes (STING), a protein located in the endoplasmic reticulum, is a key mediator of innate immune response to non-CpG cytosolic DNA. Intact STING has been shown to be involved in the induction of the innate antitumor response in Glioblastoma (GBM). Upon sensing cytosolic dsDNA, the STING pathway is activated, resulting in phosphorylation of interferon regulatory factor 3 (IRF3), which

leads to the induction of a type I interferon response, essential for innate immunity. Recent evidence shows the STING pathway is dysregulated in other cancers. METHODS: Western analysis on several glioblastoma derived cell lines, showed that STING was present at variable levels in 6 out of 7 cell lines. In addition, the dsDNA sensor cGAS, a protein upstream of STING, was expressed in only 3 out of 7 cell lines. Cell lines were treated with a variety of innate agonists including CpG free dsDNA construct to test the activation of the STING pathway. We observed a variable response to the dsDNA stimulus. The M059J cell line exhibited an intact STING pathway with activation of pIRF3 and pSTAT1 (a marker of IFN production). Both LN18 and T98G cell lines failed to activate pIRF3 and pSTAT1, whereas A172 cells showed activated pSTAT1 in the absence of pIRF3. CONCLUSION: This data suggests the STING Pathway is dysregulated in glioblastoma cell lines and highlights a potential mechanism of evasion of innate immune signaling in glioblastoma. Ongoing studies will examine the mechanism of this dysregulation and its impact on innate therapies such as PVSRIPO.

IMMU-32. RETARGETING IMMUNOEDITED GLIOMA ESCAPE VARIANTS WITH ADOPTIVE CELLULAR THERAPY

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INTRODUCTION: Adoptive T cell immunotherapy (ACT) leads to extension of median survival and long-term cures of treatment-resistant models of malignant glioma, medulloblastoma, and pontine gliomas. While very promising, tailored strategies to overcome ACT failure are needed. Therefore, we studied the tumors and adoptively-transferred T cells of KR158B glioma-bearing mice after they escaped ACT. The remaining T cells recognized the original KR158B tumor; however, the escaped tumor displayed marked immunoediting rendering it distinct from original tumor. We anticipated that successful strategies would need to be tailored to the new antigenic signature and therefore studied the HYPOTHESIS: That *ex vivo*-expanded T cells specific for glioma escape variants could successfully retarget immunoedited gliomas, despite the loss of primary antigens. METHODS: We performed RNAseq on normal brain, primary KR158B and GL261 gliomas, and tumor escape variants from KR158B (TOGA1.1/1.2) to analyze gene expression changes after ACT. We used restimulation co-cultures of T cells with tumor targets and IFN-g ELISAs to determine T cell activation. RESULTS: RNAseq showed parental tumors KR158B and GL261 share many differentially-expressed genes compared to normal brain. The escape variant TOGA1.1 demonstrated loss of ~80% of the genes that were upregulated in the KR158B and GL261 tumors. When we generated TOGA1.1 or TOGA1.2-specific T cells, they efficiently recognized TOGA1.1 or TOGA1.2 tumors but not primary KR158B or the opposite glioma escape variant. Additionally, T cell receptor (TCR)-Vb spectratyping prior to restimulation determined that distinct TCR-Vb families recognized parental tumors and TOGA1.1 and 1.2. CONCLUSIONS: Glioma escape variants are antigenically-distinct from primary tumors and other escape variants but retain expression of immunogenic antigens. Generation of ACT with specificity for escape variants provides exquisite antigen-specific recognition of the immunoedited tumors. These studies suggest feasibility of repeated treatment of tumors that have escaped ACT with generation of new, activated T cells targeting immunoedited gliomas.

IMMU-33. TARGETED IMMUNE CHECKPOINT INHIBITORS FOR INTRATUMORAL DELIVERY IN GBM

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T cells infiltrate most of solid tumors, however, in glioblastoma (GBM) the presence of these cells is heterogeneous. Using CD3 staining, we find areas of GBM tumors with very few, if any, of these immune cells or few areas that have their dense presence. Immune checkpoint inhibitors (ICI) have yet to produce clinically meaningful responses in patients with GBM. The lack of responses may be related to the limited overall presence of T cells in GBM or inefficient intratumoral trafficking of peripherally ICI-activated cells. In our strategy to make ICI more specific and more accessible to GBM tumors we redirect them to GBM tumor cells to deliver them loco-regionally. IL-13.E13K is a high affinity mutant of interleukin 13 (IL-13), preserving the binding to the cytokine's receptor alpha 2 (IL-13R2A), which is highly overexpressed on GBM cells. We have constructed two recombinant antibodies. The first consists of a single chain Fv (scFv) region of anti-CTLA-4 antibody, combined with an Fc region of human IgG1 and with IL-13.E13K (termed αCTLA4-Fc-IL-13.E13K). The second consists of the same components but with anti-PD-1 scFv instead of the αCTLA-4 (termed αPD1-Fc-IL13.E13K). Both fusion protein ICIs demonstrated competition for the IL-13R2A. In addition, the redirected immune checkpoint inhibitor αCTLA4-Fc-IL13.E13K demonstrated similar binding to CTLA-4 as did αCTLA4 antibody with Kd values in the low nM range. The αPD1-Fc-IL13.E13K, also demonstrated similar binding affinity to PD-1 as did αPD1 antibodies with Kd values also in the low nM range. Thus, αCTLA4-Fc-IL13.E13K and αPD1-Fc-IL13.E13K redirected to tumor ICI have a strong affinity for the IL-13R2A while continuing to demonstrate strong affinities for CTLA-4 and PD-1. These constructs in conjunction with other immune system stimulations like cytotoxic therapies have the potential to be effective in facilitating the interaction between T cells and GBM tumor cells directly in a tumor microenvironment.

IMMU-34. A BALANCED TRYPTOPHAN DIET LEADS TO MAXIMAL IMMUNOTHERAPEUTIC EFFICACY IN GLIOBLASTOMA MODELS

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INTRODUCTION: Immunotherapy has failed to improve glioblastoma (GBM) patient survival in all Phase III clinical trials to-date. A potential mechanism contributing to treatment failure, is based on our recent work showing that, GBM-infiltrating T cells directly increase the intratumoral expression of indoleamine 2,3 dioxygenase 1 (IDO1) (Zhai et al., 2017; CCR): an immunosuppressive enzyme that metabolizes tryptophan (Trp); an essential amino acid only provided to the body by dietary intake. Coincidently, we recently discovered that a substantial proportion of syngeneic mice with intracranial GL261, experience long-term survival when administered radiotherapy (RT) with concurrent PD-1 and IDO1 enzyme inhibitors (Ladomersky et al., 2018; CCR). Since the duration and penetration of pharmacologic IDO1 inhibition is variable, we questioned whether dietary Trp supplementation would influence efficacy of immunotherapy against GBM. METHODS: Mice were placed on ad libitum dietary formulations containing 0%, 25%, 100%, 200%, and 500% normal daily Trp, three days prior to the intracranial-injection (ic.) of 2 × 105 GL261. At fourteen days post-ic., animal subjects enrolled on each diet were treated with: (i) IgG control Ab; or (ii) the simultaneous combination of 2Gy whole brain radiotherapy for five days, four doses of PD-1 mAb (J43) every three days, and 100mg/kg IDO1 enzyme inhibitor (BGB-5777), BID, for four weeks (n=10/group). RESULTS: Mice receiving normal (100%) dietary Trp achieve a long-term survival benefit (3/10; P<0.001). In contrast and unexpectedly, both the depletion and saturation of dietary Trp, has negative effects on immunotherapeutic efficacy. Strikingly, although withholding dietary Trp decreases serological Trp levels (P<0.05), CNS parenchyma Trp concentrations remain stable, regardless of low-absent dietary Trp and peripherally-circulating levels. CONCLUSIONS: These data support that, IDO1-mediated immunosuppression is not overcome by supplementing or depleting the diet of Trp. Moreover, it suggests that a balanced diet, with normal Trp intake, provides maximal efficacy for supporting immunotherapy against malignant glioma.

IMMU-35. PSYCHOSOCIAL STRESS NEGATIVELY IMPACTS IMMUNOTHERAPY IN IMMUNOCOMPETENT MODELS OF GLIOBLASTOMA

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INTRODUCTION: Recent Phase III clinical trial findings indicate a failure of anti-PD-1 mAb (nivolumab) monotherapy to increase overall survival (OS) for recurrent glioblastoma (GBM) patients. Supportingly, we previously demonstrated that, while neither mono- nor dual-therapy with anti-PD-1, radiotherapy, or an IDO1 inhibitor, leads to long-term survival, the trimodal combination of all three durably increases survival in ~40% of mice with well-established, intracranial, syngeneic GL261. A concern arose, however, as to why ~60% of subjects always succumb to brain tumor, regardless of treatment. Psychological stress is a well-known modifier of the immune response, although the impact of psychosocial stressors on immunotherapeutic effectiveness have yet to be investigated in GBM. Because single-mouse housing induces chronic stress, we wondered if psychological factors diminish immunotherapeutic efficacy. METHODS: C57BL/6 mice were housed singly (n=10) or at five mice/cage (normally; n=10) for one week, prior to the intracranial injection (ic.) of 2 × 105 GL261 cells. At fourteen days post-ic., subjects were treated with trimodal immunotherapy as previously reported (Ladomersky et al., 2018; CCR). RESULTS: Trimodal treatment leads to a median OS of 17.5 days in singly-housed subjects, which is significantly decreased as compared to the 29 day OS for normally-housed subjects (P=0.037). Strikingly, long-term survivors were only observed in the normally-housed group. Subjects were treated with RT

and PD-1/IDO1 inhibitors, with and without the anxiety inhibiting pharmacologic, propranolol. While there was no difference in OS between subjects treated with IgG control or propranolol, alone, 60% of mice treated with all four agents survived long-term, whereas only 30% survived long-term with trimodal immunotherapy, alone (P=0.001). CONCLUSIONS: Given the association between higher anxiety levels, and an increased mortality rate among cancer patients in the U.S., these data suggest that, inhibiting stress-signaling pathways may synergize to improve immunotherapeutic efficacy, specifically improved likelihood of sustained response, for patients with malignant glioma.

IMMU-36. IMMUNE RESPONSES IN CANINE GLIOMAS ARE ENRICHED AT THE INFILTRATING EDGE OF ASTROCYTOMAS

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BACKGROUND: Immune competent spontaneously-arising gliomas in canines could be used to robustly vet immune therapeutic strategies, yet the immune composition in these CNS tumors has not been well characterized. Furthermore, few studies have examined the differences in immune reactivity between different areas of the tumor microenvironment such as the hypoxic core or the infiltrating edge. METHODS: Immunohistochemistry was used to analyze the distribution of CD3+, CD4+, and CD49a (tissue-resident memory) T cells, CD14+ monocytes, and IBA-1+ macrophages/microglia (incorporating their skew to M2 macrophages based on CD163 expression) in various tumor compartments including at the invasive edge and within the tumor or necrotic center. Tissue segmentation of canine oligodendrogliomas (n=4) and astrocytomas (n=7) was performed at 20x (0.75 NA) magnification using Perkin Elmer Vectra 3 and InForm software (Ver 2.2). Bayesian multilevel logistic regression was used to assess the immune reactivity in the various tumor compartments. RESULTS: Among the various immune populations, canine gliomas were most populated with macrophages, which constituted between 20%-30% of the total cellular population—similar to human gliomas. The macrophages were enriched at the invasive edge (OR, 1.63; 95% CI, 1.61 to 1.65). Monocytes were the next most frequent immune cell population followed by CD3, CD4, and CD49a, with all the T-cell types being rare in all tumor compartments. CD3 (OR, 1.86; 95% CI, 1.01 to 3.87), CD4 (OR, 6.2; 95% CI, 1.22 to 200), and CD163 (OR, 2.75; 95% CI, 1.12 to 37.17) at the invasive edge and CD49a (OR, 1.99; 95% CI, 1.09 to 5.19) within the tumor were more common in astrocytomas than oligodendrogliomas. The necrotic core was almost exclusively infiltrated with macrophages and monocytes. CONCLUSION: Astrocytic tumors are more immunogenic than oligodendrogliomas in canine models and are highly enriched with macrophages that predominate at the infiltrating edge.

IMMU-37. VEMURAFENIB ENHANCES THE CYTOTOXICITY OF NY-ESO-1 TCR ENGINEERED T-CELLS FOR BRAF MUTANT BRAIN TUMORS

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INTRODUCTION: Missense BRAF mutations are found in a significant portion of pediatric brain tumors, leading to constitutive MAPK pathway activation. By combining immunotherapy with BRAF targeted therapies like vemurafenib, an opportunity might exist to mitigate innate cellular immune escape mechanisms. We hypothesize that vemurafenib is an immunosensitizing agent that induces MAPK pathway de-regulation and sensitizes tumors to T-cell mediated lysis. METHODS: We treated a human glioma cell line that carries a BRAF V600E mutation (DBTRG-05MG) with decitabine to uncover an antigenic target for NY-ESO-1 transduced T-cells. Tumor cell lysis was assessed using the xCELLigence Real-Time Cell Analyzer system. The Seahorse XF Extracellular Flux Analyzer was used to measure bioenergetic pathway fluctuation with BRAF inhibition. RESULTS: Cytotoxicity data demonstrated a dose-dependent enhancement of tumor cytolysis throughout the 48-hour assay that was not attributable to BRAF inhibition alone. With one effector cell per tumor cell, a 19.95% increase in lysis was found at three hours when cells were treated with the IC20 concentration of vemurafenib versus those untreated (*P= .0028, Student's t-test). With 2.5 effector cells per tumor cell, cytolysis increased by 25.2% (*P= .0098), and by 40.52% (*P= .0081) with 5 effector cells per tumor cell. Vemurafenib treatment of tumors induced a reduction in the basal, ATP-linked, and maximal oxygen consumption rate (OCR), whereas T-cells co-cultured with vemurafenib treated tumor cells displayed an increase in aerobic and glycolytic metabolism. CONCLUSIONS: These results support an innovative, feasible treatment for BRAF mutant tumors. In a competition for metabolic fuel, the cytotoxic advantage for T-cells may be explained by reduced tumor consumption and increased availability of metabolic substrates in the tumor environment. Using the Nanostring Pathways Panel, we are also seeking to identify how vemurafenib induces oncogenic pathway deregulation, which may provide insight on plausible treatments for metabolically abnormal tumors.

IMMU-38. TARGETING HYPOXIA DOWNSTREAM SIGNALING PROTEIN, CAIX FOR CAR-T CELL THERAPY AGAINST GLIOBLASTOMA (GBM)

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BACKGROUND: The need for novel therapies for GBM treatment is well established. While chimeric antigen receptor (CAR)-engineered T cells represent a promising cancer treatment modality, highly expressed unique cell surface antigens are essential. We postulated that tumor-specific surface antigens related to hypoxia signaling may have potential. Carbonic Anhydrase IX (CAIX) is active in settings of unbalanced pH driving proton transport to the extracellular medium in response to decreased oxygen tension. We provide evidence that CAIX is highly expressed in GBM and thus a potentially suitable target which we investigated using CAIX-specific CAR-T cells against GBM cells. METHODS: Patient GBM tissues were evaluated for CAIX protein expression. Antitumor CAIX-specific CAR T cells were developed and tested against four GBM cell lines: U251, LN 229, T98G, and A172. Cytotoxicity of CAIX CAR-T cells was assessed using the levels of IFN-γ, TNF-α, and IL-2 released when co-cultured with tumor cells. Direct intra-tumor injection of CAR-T cells into intracranial GBM xenograft mouse model was used for in vivo testing. RESULTS: We confirmed high expression of CAIX in of human GBM. The viral construct of CAIX-specific CAR-T cells generated was tested against GBM cell lines. *In vitro*, CAIX-specific CAR-T cells displayed enhanced GBM cells cytolysis and increased IFN-γ, TNF-α, and IL-2 production. The CAR-T cells showed a specific CAIX-dependent recognition of GBM cells. Direct intra-tumor injection of the CAR-T cells into an intracranial GBM xenograft mouse model efficiently suppressed the growth of GBM cells and significantly prolonged mouse survival. In approximately 20%, CAIX specific CAR-T cells completely eradicated GBM tumors. CONCLUSIONS: The present study demonstrates the specificity of CAIX under hypoxic conditions in GBM. The results also show that CAIX represents a viable target for CAR-T cells and may be a promising strategy to treat GBM.

IMMU-39. FIRST-IN-KIND T CELLS CARRYING A CHIMERIC ANTIGEN RECEPTOR AGAINST AN EXTRACELLULAR MATRIX PROTEIN TARGET GLIOBLASTOMA CELLS AND SHOW ANTI-TUMOR EFFICACY

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Immunotherapies using engineered T lymphocytes expressing chimeric antigen receptors (CAR-T cells) are showing great promise against glioblastoma but are still limited by the escape of antigen-deficient clones and by inefficient latching of the T cells to target cells surrounded by extracellular matrix (ECM). Here, we tested the hypothesis that an antigen localized in the pericellular ECM could be sufficient to elicit CAR-T activation and cytotoxicity at sufficiently close distance to their targets, overcoming in part tumor heterogeneity and the dependence for perfect binding to the target cells. Using our recently developed function-blocking antibody against the ECM protein fibulin-3 produced by GBM cells, we generated a recombinant scFv fragment that can recognize and block the pro-tumoral functions of this protein. This scFv was then used to generate a second generation anti-fibulin-3 CAR (fib3CAR) stably transduced in T cells. We confirmed that fib3CAR-T cells were activated both by soluble fibulin-3 as well as fibulin-3-secreting GBM cells. This activation involved: a) Increased release of interferon-gamma; IL2; perforin, and granzyme; b) Increased proliferation of the CAR-T population; and c) Specific cytotoxicity against co-cultured GBM cells and other fibulin-3-secreting cells but not against control

cells. Activation of fib3CAR-Ts was dependent on canonical NFAT signaling and was blocked by NFAT-inhibition. Moreover, time-lapse microscopy showed tracking and latching of GBM cells by fib3CAR-Ts but not by control T cells. Nude mice carrying GBM stem cell-derived tumors (both sub-cutaneous and orthotopic) were treated with intratumoral injections of fib3CAR-Ts. The results from this treatment showed tumor reduction, persistence of T cells in the tumor mass, lack of antigenic escape, and significant prolongation of survival. Fib3CAR is the first CAR developed to recognize an antigen localized in the ECM, which raises novel options for immunotherapies against the pericellular microenvironment in malignant gliomas and other solid tumors.

IMMU-40. SINGLE-CELL LEVEL COMPARISON OF HISTOPATHOLOGY AND SINGLE-CELL RNA-SEQ DATABASES BETWEEN IDH-MUT AND –WT GLIOBLASTOMAS REVEALS DISTINCT INNATE IMMUNE MICROENVIRONMENTS THAT CAN BE EXPLOITED FOR THERAPEUTIC GAIN

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BACKGROUND: Most immune cells in the glioblastoma (GBM) microenvironment are microglia and macrophages (MMs), but they are poorly understood. We sought to characterize these innate immune cells in human untreated IDH-WT and rare IDH-MUT GBM tissue to elucidate differences underlying their disparate prognoses relevant to immunotherapy design. METHODS: An in-house automated segmentation protocol that quantifies at the single-cell level was used to analyze newly diagnosed human GBM (9 IDH-WT, 4 IDH-MUT). Three large sections (3-8mm in diameter) were quantitated to capture potential spatial heterogeneity. Expression of CD68, HLA-A/B/C, TNFa, CD163, IL10, TGFB2, Iba1 intensity, and surface area were enumerated and combined into an activation profile in Iba1+ cells (MMs). Results were validated with flow cytometry. Human IDH-MUT (GSE89567) and –WT (GSE84465) single-cell RNA-seq databases were then compared using novel bioinformatics techniques to affirm results. RESULTS: MM content is drastically reduced in IDH-MUT compared to –WT GBMs (4.9 ± 1.4% vs. 37.2 ± 7.3% of all GBM cells, respectively; p=0.0154). Surprisingly, a large range of MM content was found in IDH-WT GBMs, from 1.6 ± 0.6% to 71.9 ± 13.4%. Positive correlation with flow cytometry corroborated these results (Pearson r=0.7296; p=0.026). Inflammatory phenotypic variability was again seen in MMs in both IDH-MUT and –WT GBMs, but IDH-MUT GBM-associated MMs were more activated/pro-inflammatory (124.5 ± 21.6 pro-inflammatory units vs. 54.0 ± 15.6 pro-inflammatory units; p=0.0265). Comparison of single-cell RNA-seq databases after normalization and dynamic pruning of hierarchical clustering dendrograms verified MMs in IDH-MUT GBMs were more pro-inflammatory, but that this was driven by anti-inflammatory macrophages in IDH-WT GBMs as opposed to microglia which were pro-inflammatory in all tumors ($p < 0.01$). CONCLUSION: This is one of the first studies to characterize MMs in untreated human IDH-MUT GBMs and identify dissimilarities to the IDH-WT innate immune microenvironment that can be targeted by immunotherapies. Also, considerable MM phenotypic heterogeneity suggests precision immunotherapy approaches are crucial.

IMMU-41. IDO1 INCREASES Treg RECRUITMENT INDEPENDENT OF TRYPTOPHAN METABOLISM IN A MODEL OF GLIOBLASTOMA

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OBJECTIVE: Glioblastoma (GBM) patients with high intratumoral IDO1 mRNA levels have an associated decrease in survival (Zhai *et al*., 2017; CCR). IDO1 is an immunosuppressive mediator that metabolizes tryptophan (Trp), and through its associated enzyme activity, has long been recognized to increase immunosuppressive $CD4^+CD25^+FoxP3^+$ Tregs. Accordingly, we previously showed that the genetic knockdown of IDO1 in murine glioma cells, suppresses intratumoral Treg recruitment and increases animal subject survival. Unexpectedly, however, IDO1 knockdown has no effect on intratumoral Trp metabolism (Zhai *et al*., 2017; BBI). Further surprisingly, we recently demonstrated that, although IDO1 overexpression increases Treg levels in brain tumors, the pharmacological inhibition of IDO1 metabolism has no effect on Treg accumulation (Ladomersky *et al*., 2018; CCR). These novel data led us to question the requirement for IDO1 enzyme activity to regulate Tregs in malignant glioma. METHODS: Lentiviral vectors encoding: (i) vector control-, (ii) wild-type (WT)-, or (iii) enzyme-null-IDO1, were created by site-directed mutagenesis in a plasmid containing WT, murine, IDO1-GFP cDNA. IDO1-deficient glioma cells from transgenic mice [$(ERT2)GFAP{\rightarrow}Cre;pTEN^{fl/fl};p53^{fl/fl};Rb^{fl/fl};IDO1^{-/-}$] were transduced with the modified plasmids. RT-PCR, Western blotting and HPLC confirmed IDO1 expression and enzyme activity, *in vitro*. Syngeneic $IDO1^{-/-}$ mice were intracranially-engrafted with 2×10^5 modified glioma cells and studied for intratumoral Treg levels. RESULTS: The substitution of IDO1 histidine 350, to an alanine (H350A), decreases IDO1 enzyme activity by 90%. Syngeneic mice with intracranial, IDO1 enzyme null (H350A) glioma, have similar intratumoral Treg levels as IDO1 WT glioma, at 25% and 26%, respectively, and is significantly increased as compared to the 5.8% Tregs in vector control glioma (P=0.015). CONCLUSIONS: Our data challenge current dogma explaining how IDO1 causes Treg accumulation, and are in-line with the recent IDO1 enzyme inhibitor-focused Phase III clinical trial failure [NCT02752074]. We are now focused on revealing the mechanism underlying IDO1-mediated immunosuppression in malignant glioma.

IMMU-42. TTFIELDS INDUCES IMMUNOGENIC CELL DEATH AND STING PATHWAY ACTIVATION THROUGH CYTOPLASMIC DOUBLE-STRANDED DNA IN GBM

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Glioblastoma (GBM) is the most common and deadliest malignant brain cancer in adults despite aggressive chemoradiotherapy. Recently, Tumor Treating Fields (TTFields) were approved in combination with adjuvant temozolomide chemotherapy for newly diagnosed GBM patients. The addition of TTFields resulted in a significant improvement in overall survival. TTFields are low-intensity alternating electric fields that are thought to disturb mitotic macromolecules' assembly, leading to disrupted chromosomal segregation, integrity and stability. In many patients, a transient stage of increased peritumoral edema is often observed early in the course of TTFields treatment followed subsequently by objective radiographic responses, suggesting that a major component of therapeutic efficacy by TTFields may be an immune mediated process. However, the mechanism underlying these observations remains unclear. A panel of GBM cell lines were treated with TTFields at the clinically approved frequency of 200 kHz using the inovitro system. Our data showed TTFields-treated GBM cells had a significantly higher rate (19.9% vs. 4.3%, p=0.0032) of micronuclei structures released into the cytoplasm as a result of TTFields-induced chromosomal instability. Nearly 40% of these micronuclei were co-localized with two upstream dsDNA sensors Interferon (IFN)-inducible protein absent in melanoma 2 (AIM2) and Cyclic GMP-AMP synthase (cGAS), compared to absence of co-localization in untreated cells. TTFields-activated micronuclei-dsDNA sensor complexes led to i) induction of pyroptotic cell death, as measured by a specific LDH release assay, and through AIM2-recruited caspase1 and cleavage of pyroptosis-specific Gasdermin D; and ii) activation of STING pathway components including Type I IFNs and pro-inflammatory cytokines in GBM cells. These results provide compelling evidence that TTFields function as an activator of the immune system in GBM, and present a strong rationale for combining TTFields with immunotherapy aimed at augmenting an anti-tumor immune response such as immune checkpoint inhibitors.

IMMU-43. RNA-MODIFIED T CELLS AS A NON-INVASIVE AND EFFICACIOUS STRATEGY TO DELIVER THERAPEUTIC MACROMOLECULES LOCALLY TO INTRACRANIAL TUMORS

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INTRODUCTION: With the presence of the blood-brain barrier (BBB), successful drug delivery to central nervous system (CNS) malignancies remains a challenge. By inducing tumor regression and potentiating immune responses, cytokines, such as granulocyte macrophage colony-stimulating factor (GM-CSF), have shown promise as a biological agent to enhance anti-tumor efficacy in preclinical studies and cancer immunotherapy trials. However, intravenous cytokine delivery has limited access to CNS, and are often associated with significant systemic side effects. To bypass limitations of systemically administered cytokines, we investigate the HYPOTHESIS that RNA-modified T cells can deliver macromolecules directly to intracranial tumors. Since activated T cells have an inherent ability to cross the BBB and lyse tumor cells, this strategy makes them an attractive biological carrier for cancer cell therapy. METHODS: Using electroporation to deliver messenger RNA (mRNA) to T cells, we evaluated GM-CSF secretion and the function of GM-CSF RNA-modified T cells. RESULTS: We demonstrated that activated T cells could be modified to secrete GM-CSF protein *in vitro*, while retaining their inherent effector functions. In a murine intracranial tumor model, GM-CSF RNA-modified

T cells effectively delivered GM-CSF to the tumor microenvironment *in vivo*, and significantly extended median overall survival with long term cures in some treated animals. Importantly, GM-CSF expressing T cells demonstrated a superior anti-tumor efficacy compared to unmodified T cells, and systemic administration of recombinant GM-CSF provided no treatment benefit when co-delivered with unmodified T cells. Such anti-tumor effects were associated with increased interferon gamma secretion locally within tumor microenvironment, and systemic antigen-specific T cell expansion within secondary lymph nodes. CONCLUSIONS: This study strongly supports the implementation of a non-invasive and efficacious strategy to deliver therapeutic macromolecules locally to invasive brain tumors, while offers potential widespread applicability for the delivery of biological agents to other CNS conditions, such as neurodegenerative or neuroinflammatory diseases.

IMMU-44. OPTICAL BARCODING TO INVESTIGATE CLONAL DYNAMICS OF GBM HIGHLIGHTS THE INTRINSIC CAPACITY OF GBM TO RE-ACTIVATE DEVELOPMENTAL GENES AND ESCAPE IMMUNE SURVEILLANCE

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The dynamic interplay of the heterogeneous nature of GBM within the microenvironment remains one of the biggest challenges and inhibits effective treatment strategies. It is still unknown how the brain microenvironment and in particular the immune cells are shaping tumor cell clonal heterogeneity and contribute to drug resistance. To model the growth dynamic of clonal GBM populations *in vivo*, we used patient derived cells lines (PDX, n = 10) as well as a syngeneic mouse line, labelled with an RGB combination of colors, allowing us to directly visualize thousands of clones. Brains were analyzed by confocal microscopy and flow cytometry, and an algorithm was developed to quantify and plot clonal heterogeneity as 3D graphs. The results demonstrated different degrees of clonal constriction and selection, depending on cell type and microenvironment. We next investigated the GBM clonal development by tracking clones applying a unique approach called optical barcoding (OBC). We were able to re-isolate subpopulations grown out from single cells in immunocompetent vs immunodeficient mice by cell sorting and to analyze specific clones by RNAseq. In the absence of immune cells, clonal constriction appears to be more random and less stringent while the recruitment of T cells, macrophages and NK cells during the early phase of tumor growth shapes tumor heterogeneity, favoring some specific clones fit to escape the immune cells. Moreover, we discovered in our PDX models that the comparison of bulk tumor expression profiles to only the fittest clones that contribute to tumor growth highlighted the specific expression of genes involved in neurogenesis and neural stem cell specification. These results indicate that clonal GBM cell expansion is not only determined by genomic alterations but also by their capacity to re-activate developmental gene expression programs.

IMMU-45. DOSE MODULATION OF TEMOZOLOMIDE HAVE DISTINCT EFFECTS ON HOST RESPONSE TO PD-1 BLOCKADE

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INTRODUCTION: Temozolomide is known to affect efficacy of immunotherapy due to effects on the tumor microenvironment and host immune cell function. The effects of temozolomide dosing on efficacy of immune checkpoint inhibition is unknown. HYPOTHESIS: Dose modification of temozolomide modulates host immunity to increase efficacy of immune checkpoint blockade in a murine syngeneic glioblastoma model. METHODS: Experiments were performed utilizing GL261 tumor bearing mice treated with standard dose (SD) temozolomide (50mg/kg x 5 days), metronomic dose (MD) temozolomide (25mg/kg x 10 days) and/or anti-PD1 antibody. RESULTS: SD temozolomide treatment resulted in greater lymphopenia and a more immunosuppressive profile compared to MD temozolomide in GL261 tumor bearing mice. SD temozolomide caused an upregulation of Tim-3, Lag-3 and PD-1 on peripheral and splenic CD4 and CD8 T cells. MD temozolomide increased PD-1 expression without concomitant Tim-3 or Lag-3 expression on CD4 and CD8 T cells. SD temozolomide also resulted in an increase in myeloid derived suppressor cells which was not observed with MD temozolomide. Moreover, antigen specific CD8 T cells were less functional as measured by IFN-gamma secretion when treated with SD temozolomide as compared with MD temozolomide. Analysis of tumor infiltrating lymphocytes also demonstrated increased exhaustion when treated with SD temozolomide compared to MD temozolomide. Combination treatment with PD-1 blockade and either MD or SD temozolomide demonstrated higher expression of checkpoints and immune exhaustion profiles in the SD temozolomide group measured by RNA sequencing. Survival analysis revealed that PD-1 blockade resulted in survival benefit in tumor bearing animals. However, SD temozolomide abrogated this survival benefit when combined with PD-1 blockade. MD temozolomide preserved the survival advantage of PD-1 blockade. CONCLUSION: Dose modification of temozolomide impacts efficacy of immune checkpoint blockade by modulating immune effector cells. Strategies to reverse T cell exhaustion induced by SD temozolomide are underway.

IMMU-46. GLIOBLASTOMA PATIENT DIAGNOSES AND IMMUNOSUPPRESSION ARE MAXIMAL DURING OLD AGE: A RANDOM COINCIDENCE, OR CAUSE AND EFFECT?

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INTRODUCTION: Immunotherapy fails to improve overall survival (OS) of adults with glioblastoma (GBM) according to recent Phase III clinical trial outcomes. Though immune checkpoint blockade improves OS among patients diagnosed with other, end-stage, non-CNS cancers (ie. melanoma, renal, etc.), there now exists a question of whether immune-based therapies hold promise for GBM. Somewhat under-appreciated is that, de novo GBM is a disease that primarily affects the elderly, with a median age of diagnosis at 64 years old. Strikingly, we recently discovered a higher mortality rate in elderly C57BL/6 mice with syngeneic, intracranial GL261, as compared to young, 6-8-week-old counterparts, after simultaneous treatment with radiotherapy and PD-1/IDO1 inhibitors (Ladomersky et al., 2018; CCR). METHODS: To follow-up the striking age-dependent decrease of immunotherapeutic efficacy, preclinically, we investigated the: (i) Surveillance, Epidemiology, and End Results (SEER) database; (ii) Broad Institute's GTex portal; and (iii) 10k Immunomes repository, to understand the relationship(s) between human GBM, human immunology and aging. RESULTS: GBM patient incidence is 3.4× higher among individuals ≥65 years old, as compared to those <65 (n=7155; P<0.0001). Unexpectedly, the rate of mortality among GBM patients ≥65 years old, is 7× higher, as compared to GBM patients <65 (n=9761; P<0.0001). Strikingly, immunosuppressive IDO1 levels increase in the normal human brain with advanced age, and is maximal among individuals aged 60–69 years old (n=1152; P<0.05). There is also a maximal incidence of circulating immunosuppressive Tregs among normal individuals aged 65–74 years old (n=578; P<0.05). CONCLUSIONS: The elderly population has a cumulative peak for indicators of immunosuppression, at the same age range as the maximal incidence of GBM patient diagnoses. We are currently pursuing how old age enhances immunosuppression, is associated with the maximal incidence of GBM diagnoses, and detracts from immunotherapy for malignant glioma, which is a clinically-relevant priority.

IMMU-47. HARNESSING ZIKA VIRUS (ZIKV) ONCOLYTIC ACTIVITY IN BRAIN TUMORS

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Glioblastoma (GBM) is a highly lethal immunosuppressive brain cancer that contains glioblastoma stem-like cells (GSCs). GSCs are at least partly responsible for tumor maintenance, progression, recurrence and resistance and are thus potential therapeutic targets. ZIKV, a flavivirus, preferentially infects and kills glioblastoma stem cells (GSCs) compared to differentiated tumor progeny and normal neural cells (Zhu, Z. et al. Zika virus has oncolytic activity against glioblastoma stem cells. J. Exp. Med.2017). We are actively investigating if ZIKV-mediated tumor clearance is dependent on the dual mechanism of action of selective tumor cell killing and the induction of systemic anti-tumor immunity. In anticipation of possible clinical use of ZIKV as a GSC therapy, we have introduced mutations in the ZIKV genome that will (1) prevent 2-'O methylation of viral RNA and render it sensitive to inhibition by IFIT1, (2) prevent the generation of non-coding subgenomic (sfRNA) viral RNA that antagonizes type I IFN immunity, (3) limit viral dissemination, and (4) make reversion to the pathogenic virus

a highly unlikely event. We are in the process of testing *in vivo* the efficacy of tumor killing and control of these modified viruses, compared to the parental control. Additionally, we are investigating the effect of ZIKV in controlling patient-derived GSCs *in vivo* using NSG (non-obese diabetic severe combined immune deficiency, IL2Rγ-null) mice. Lastly, we will present preliminary data on ZIKV treatment followed by immune checkpoint blockade. These findings will further advance the potential use of ZIKV in GBM oncolytic virotherapy.

IMMU-48. GLIOMA IMMUNE PROFILING REVEALS UNIQUE IMMUNE THERAPEUTIC OPPORTUNITIES

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INTRODUCTION: There is a rapidly evolving portfolio of immune therapeutic modulators, but the relative incidence of immune targets in human gliomas is unknown. To prioritize available immune therapeutics, comprehensive immune profiling across glioma grades was conducted. METHODS: CD4+ and CD8+ T-cells and CD11b+ monocytes/macrophages were isolated from the blood of healthy donors (n= 6) and the blood and tumors of newly diagnosed and recurrent grade II (n= 7), III (n= 6) and IV glioma patients (n= 12), and profiled for the expression of 30 immune modulatory targets. RESULTS: In CD4+ and CD8+T-cells, PD-1 and the adenosine pathway showed high expression levels in tumor-infiltrating lymphocytes (TILs) across glioma grades and treatment states. The mean fluorescent intensity (MFI) of A2aR was appreciably upregulated in the TIL and in the CD11b+ glioma-infiltrating monocytes/macrophages (GIM) compared to healthy donors (CD4+ p= 0.0201; CD8+ p= 0.0233; CD11b+ p= 0.0009) and matched peripheral blood (CD4+ p= 0.0054; CD8+ p= 0.019; CD11b+ p< 0.0001). LAG3 and TIM3 TIL expression frequency was low and not associated with glioma grade. TIGIT CD8+TIL were more frequent in high-grade gliomas (grade III and IV). CD39 was upregulated in the TIL compared to healthy donors (CD4+ p=0.0032; CD8+ p=0.0006) and matched peripheral blood (CD4+ p= 0.0002; CD8+ p< 0.0001). B7-H3 (p= 0.0167) and Galectin-9 GIM expression (p= 0.0042) are frequently upregulated in glioblastoma. Regardless of glioma grade, the T-cell co-stimulatory molecule CD86 is down-regulated in the GIM (p= 0.0019). Many immune modulatory markers were not frequently upregulated but exceptionally high in individual patients. CONCLUSION: Predicated on expression frequency, immune therapeutics targeting PD-1 and the adenosine pathway would be applicable to most glioma patients regardless of grade or prior treatment status. Clinical trials considering the use of other immune targets, such as LAG3, TIM3, TIGIT, BTLA, B7-H3, and Galectin-9 need to consider a companion biomarker for enrollment enrichment.

IMMU-49. CYTOTOXIC T CELLS AND THEIR ACTIVATION STATUS ARE INDEPENDENT PROGNOSTIC MARKERS IN MENINGIOMAS

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Although clinically aggressive meningiomas (MGMs) are rare tumors, more effective therapies are urgently needed. As a prerequisite for successful immunotherapy we determined the infiltration and activation status of T cells in primary (p-) and recurrent (r-) MGMs and their impact on survival. Presence of tumor-infiltrating lymphocytes (TILs) was assessed in a large, clinically well-annotated study sample of 202 cases (n=123 pMGMs, and n=79 rMGMs) with a substantial proportion of higher-grade MGMs (n=43 WHO°I, n=97 WHO°II, n=62 WHO°III). TIL quantification was performed by a semi-automated analysis on whole tissue sections stained by multi-color immunofluorescence for CD3, CD8, FOXP3, and PD-1. Ranging from 0.01–24.88 %, median T cell infiltration accounted for 0.59 TILs per total cell count (TCC). Although there were no significant changes for the proportion of helper and cytotoxic T cells in pMGM of different WHO grades, higher numbers of cytotoxic T cells were associated with an improved progression-free survival (PFS) independent of prognostic confounders. rMGM were characterized by significantly lower numbers of TILs in general (median: 0.33 % per TCC), helper and cytotoxic T cells and a significant increase of regulatory T cells. As for the activation of TILs, about one third expressed the immune checkpoint molecule PD-1 and predominantly were CD8-positive. We observed a significant WHO-dependent decrease of PD-1$^+$/CD8$^+$ TILs which in univariate and multivariate analyses were associated with a poorer PFS. In line with these findings proportions of PD-1$^+$/CD8$^+$ TILs were significantly lower in rMGM arguing for PD-1 as an activation rather than an exhaustion marker. In summary, we were able to identify intratumoral cytotoxic TILs as well as of PD-1-expressing cytotoxic TILs as novel and easy applicable biomarkers for a better survival, which might facilitate the selection of patients who could benefit from immunotherapeutic approaches and mandates for an intervention in primary rather than recurrent tumors.

IMMU-50. THE IMMUNE LANDSCAPE OF BLOOD DENDRITIC CELLS IN GLIOBLASTOMA MULTIFORME: IMPLICATIONS FOR DC VACCINATION COMBINED WITH CHECKPOINT INHIBITION

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Therapeutic dendritic cell vaccination for GBM using primary blood dendritic cells (BDC) has the potential to improve response rates compared with more commonly used monocyte-derived dendritic cells due to their superior antigen-presentation and migration. Combination of vaccination with immune checkpoint inhibitors such as anti-PD-1 antibodies may remove tumour-associated immune suppression and directly enhance DC function. GBM is associated with systemic as well as local immune suppression. Therefore it is critical to understand the immune landscape and effect of current treatments on DC function and modulate this to successfully translate vaccination into patients. To that end, we firstly compared the expression of immune checkpoint molecules (PD-1, CTLA-4, TIM-3, LAG-3, ICOS, CD27 axes) on BDC (DC1, DC 2/3, DC6) and lymphocyte subsets (CD3+, CD4+, CD8+, CD56+, Treg) from newly diagnosed or currently treated GBM patients (GBM) with age-matched healthy donors (HD) using flow cytometry. This analysis revealed that the major BDC subsets were identifiable in HD and GBM but were affected by both corticosteroids and concurrent chemoradiation. Furthermore, phenotypic differences exist between HD and GBM, most notably in expression of PD-L2 by BDC. We tested the functional capacity of CD1c+ BDC from HD and GBM patients. After optimizing the maturation process of CD1c+ BDC, their ability to initiate polyclonal immune responses was determined using one-way mixed leucocyte reactions. To further augment vaccine efficacy and overcome tumour-associated immune suppression, we combined this with immune checkpoint inhibitors such as anti-PD-1 and anti-CTLA-4 antibodies. BDC were loaded with the glioma antigen, CMV-pp65 protein to demonstrate the combined effect of immune checkpoint blockade on cytokine production by intracellular staining, antigen-specific T cell expansion and cytotoxicity. DC vaccination in GBM with BDC is a feasible, rational combination with immune checkpoint inhibitors which enhances cytotoxicity in an antigen-specific fashion.

IMMU-51. THE COMBINATION OF CCR2 ANTAGONIST AND PD-1 BLOCKADE PROLONGS SURVIVAL IN IMMUNE CHECKPOINT INHIBITOR RESISTANT GLIOMAS

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INTRODUCTION: Immuno-therapy directed at the PD-1/PD-L1 axis has produced significant treatment advances in various human cancers. Unfortunately, this progress has not extended to glioblastoma, with recent clinical trials failing to show efficacy of anti-PD-1 monotherapy in recurrent tumors. Commonly employed murine glioma models exhibit varied responsiveness to anti-PD-1 monotherapy, e.g. GL261 gliomas are sensitive while KR158 tumors are resistant. Previously, we reported that combining PD-1 blockade with either chemokine receptor CCR2 deficiency or a CCR2 antagonist improved survival over anti-PD-1 monotherapy in GL261 gliomas. This was associated with reduced numbers of MDSCs within tumors. The current study evaluated the combination of PD-1 blockade and a novel CCR2 antagonist in anti-PD-1 resistant gliomas. OBJECTIVE: Determine if combination anti-PD-1/CCR2 antagonist therapy is an effective treatment in anti-PD-1 insensitive gliomas. METHODS: Overall survival and immune cell characteristics were determined in KR158 and 005GSC gliomas

established in either CCR2 deficient or wild type mice treated with CCR2 antagonist (CCX872) and/or PD-1 blockade. RESULTS: CCR2 deficiency unmasked an anti-PD-1 survival benefit in KR158 glioma-bearing mice. CCX872 increased median survival as a monotherapy in KR158 tumor-bearing animals, and significantly increased median and durable overall survival when combined with anti-PD-1. In 005GSC tumors, the combination of CCX872 and anti-PD-1 prolonged median survival time. Increases in overall $CCR2^+$ cells and $CD11b^+/Ly6C^{hi}$ myeloid derived suppressor cells (MDSC, known to be $CCR2^+$) were evident in the bone marrow of CCR2-deficient mice. Additionally, these mice exhibited decreased MDSCs within established gliomas. The data demonstrate CCX872/anti-PD-1 synergize to increase survival in clinically relevant glioma models via reduced MDSC infiltration, resulting in a tumor microenvironment favorable for anti-PD-1 efficacy. CONCLUSION: The combination of CCX872 and anti-PD-1 is effective in clinically relevant murine glioma models, providing a basis on which to progress this novel combinatorial treatment toward early phase human trials.

IMMU-52. TUMOR TREATING FIELDS (TTFIELDS) INDUCE IMMUNOGENIC CELL DEATH RESULTING IN ENHANCED ANTITUMOR EFFICACY WHEN COMBINED WITH ANTI-PD-1 THERAPY

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Tumor Treating Fields (TTFields) are an effective anti-neoplastic treatment modality delivered via noninvasive application of low intensity (1–3 V/cm), intermediate frequency (100–300 kHz), alternating electric fields. TTFields are employed as a regional treatment modality using insulated transducer arrays applied to the skin, with the intent to kill tumor cells and reduce local recurrence. This therapy is approved for the treatment of patients with glioblastoma. Previous investigations have shown that TTFields disrupt microtubules and septin filaments, both of which govern key processes in mitosis. The outcomes of mitosis under TTFields application include abnormal chromosome segregation, which trigger different forms of cell death. In this study we evaluated whether TTFields-induced cell death can be immunogenic. We demonstrate that cancer cells that die during TTFields application exhibit endoplasmic reticulum (ER) stress leading to calreticulin translocation to the cell surface and release of damage-associated molecular patterns including the chromatin-binding protein HMGB1 and adenosine triphosphate. Further, we show that TTFields-treated cells promote phagocytosis by dendritic cells (DCs) and maturation of DCs under co-culture conditions. In vivo, the combined treatment of lung tumor-bearing mice with TTFields in combination with the immune checkpoint inhibitor anti-PD-1, significantly improved therapeutic efficacy compared to the control group or TTFields and anti-PD-1 alone. Significant increase in the number of tumor infiltrating immune cells was observed in the TTFields plus anti-PD-1 group. These infiltrating cells, specifically macrophages and DCs, demonstrated upregulation of surface PD-L1 expression. Correspondingly, cytotoxic T-cells isolated from these tumors have shown higher levels of IFN-γ production relative to untreated mice. Collectively, our results suggest that TTFields application induces both ER stress and autophagy, resulting in immunogenic cell death. Combining TTFields with anti-PD-1 may therefore achieve tumor control by further enhancing antitumor immunity.

IMMU-53. IMPACT OF TUMOR-TREATING FIELDS (TTFIELDS) ON THE IMMUNOGENICITY OF GLIOMA CELLS

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BACKGROUND: TTFields have been tested in phase III trials in recurrent and in newly diagnosed glioblastoma patients. However, the impact of TTFields on the tumor microenvironment and on interactions between tumor cells and the immune system remain poorly characterized. Thus, we investigated the interaction of TTFields with molecules involved in immune responses as well as drugs that may modulate immune cell activity such as dexamethasone. METHODS: Natural killer group 2 member D (NKG2D) ligand mRNA expression was determined by RT-PCR. NKG2D ligand, MHC class I and class II, and calreticulin (CRT) protein levels were assessed by flow cytometry and high-mobility group protein 1 (HMGP-1) levels by immunoblot analysis. Immune cell-mediated glioma cell lysis was determined using a flow cytometry-based cytotoxicity assay. RESULTS: We noticed increased NKG2D ligand expression and enhanced NK cell-based killing of glioma cells exposed to TTFields while MHC class I and class II expression remained unaltered. The observed increase in calreticulin exposure upon TTFields treatment may point to enhanced immunogenicity of dying glioma cells, known as immunogenic cell death. CONCLUSION: Exposure to TTFields may change the immunogenicity of glioma cells which might be exploited for combinatorial approaches with immunotherapeutic agents. Better understanding of the mode of action of TTFields is important for further clinical development.

IMMU-54. THE ONCOMETABOLITE R-2-HYDROXYGLUTARATE SUPPRESSES THE INNATE IMMUNE MICROENVIRONMENT OF IDH1-MUTATED GLIOMAS VIA ARYL HYDROCARBON RECEPTOR SIGNALING

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BACKGROUND: IDH1-mutated gliomas are associated with less abundant and phenotypically skewed innate and adaptive immune cell infiltrates compared to IDH1 wild-type tumors. Despite this, the most frequent mutation, IDHR132H, represents a clonal shared neoantigen and mutations in IDH are associated with a more favorable prognosis. While the tumor cell-intrinsic consequences of the oncometabolite R-2-hydroxyglutarate (R-2-HG) accumulating in IDH1-mutated gliomas as a result of a neomorphic enzymatic function, are well-characterized, potential direct paracrine effects of R-2-HG influencing the glioma immune microenvironment remain incompletely understood. METHODS AND RESULTS: By means of comprehensive analyses of expression datasets from human gliomas and syngeneic murine tumor models as well as transporter studies we demonstrate that R-2-HG is imported by both microglia and macrophages via SLC family transporters and suppresses their function in a paracrine manner. Functional analyses of microglia and macrophages indicate an R-2-HG-driven induction of tolerogenicity as evidenced by accumulation of IL10 and TGFβ and suppression of MHC-II expression, which results in impaired activation of antigen-specific T cells and activation of immune checkpoint molecules. Multi-level signature profiling of human tumor-infiltrating as well as primary immune cells was complemented by reporter gene assays and pathway analyses and revealed that R-2-HG activates the cytosolic transcription factor aryl hydrocarbon receptor (AHR), a key immunomodulatory target of immunosuppressive tryptophan metabolism. Functional relevance of R-2-HG-mediated, AHR-driven impairment of myeloid cell immunity was demonstrated *in vivo* by pharmacological AHR inhibition, increasing the efficacy of checkpoint blockade. CONCLUSION: R-2-HG impairs antitumor immunity in IDH1-mutated gliomas by activating the AHR in innate immune cells, thus suppressing the innate immune microenvironment by compromising antigen presentation and activation of antigen-specific T cells. This, together with recent findings on inhibitory effects on T cell immunity, represents a novel mechanism of immune evasion of an immunogenic driver mutation and opens a novel therapeutic approach to IDH1-mutated gliomas.

IMMU-55. IMMUNOMODULATORY IL-7 AND IL-12-EXPRESSING MSCs INDUCE LONG-TERM SURVIVAL AND IMMUNITY IN SYNGENEIC INTRACEREBRAL GLIOBLASTOMA MODELS

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Mesenchymal stem cells (MSCs) show an inherent brain tumor cell tropism that can be exploited for targeted delivery of therapeutic genes to invasive glioma. We assessed whether a motile MSC-based local immunomodulation is able to overcome the immunosuppressive glioblastoma microenvironment and to induce an antitumor immune response. Apceth-301 is a cell-based immunotherapy consisting of MSCs which are genetically modified to co-express high levels of IL-12 and IL-7. In vitro characterization demonstrated increased T-cell activation, as measured by increased secretion of IFNg and TNFa, and promoted NK cell mediated killing of GBM cell lines in co-culture assays. Therapeutic efficacy was assessed in two immunocompetent orthotopic C57BL/6 glioma models using GL261

and CT2A. Intratumoral administration of MSCIL7/12 induced a significant tumor growth inhibition and displayed tumor necrosis MR imaging. Notably, up to 50% of treated mice survived long-term. Re-challenging of survivors confirmed long-lasting tumor immunity. Immunomodulatory effects were assessed by immunehistology and multicolor flow-cytometry to comprehensively profile immune activation of tumor-infiltrating lymphocytes (TIL). Local treatment with MSC-IL12/7 was well tolerated and led to a significant inversion of CD4+/CD8+ T-cell ratio with an intricate predominantly CD8+ T-cell mediated anti-tumor response. T-cell receptor sequencing demonstrated increased diversity of TILs in MSCIL7/12-treated mice, indicating a broader tumor-specific immune response with subsequent oligoclonal specification during generation of long-term immunity. Local MSC-based immunomodulation is able to efficiently alter the immunosuppressive microenvironment in glioblastoma. The long lasting therapeutic effects warrant a rapid clinical translation of this concept and have led to planning of a phase I/II study.

IMMU-56. CXCR1/2 MODIFIED CARs CO-OPT RADIATION-INDUCED IL-8 FOR ENHANCED CHEMOTAXIS OF THE CAR T CELLS AND MAXIMAL ANTI-TUMOR EFFICACY

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BACKGROUND: Glioblastomas (GBM) are heterogeneous brain tumors with capacity for malignant propagation. The treatment options are confined to surgery and chemo/radiation with marginal efficacy, thus, identifying novel markers/targets and development of novel targeted therapeutics is requisite. Cancer immunotherapy provides a unique tumor-specific therapy with exquisite precision; however, very few glioma-specific targets have been discovered. We demonstrated that CD70 is ectopically overexpressed on adult and pediatric gliomas. The expression on glial tumor cells is a markedly poor prognostic marker, mediates the recruitment of immunosuppressive myeloid cells through upregulated chemokine networks in GBM tumor cells, and results in the deletion of CD8+ cytotoxic lymphocytes. As such, this molecule plays an important role in glioma-induced immunosuppression and makes the elimination of CD70+ tumor cells an attractive axis for therapeutic targeting. OBJECTIVE: To address the key obstacle in treating solid tumors using CAR-T cells, we have developed CD70 CAR-2.0 to co-opt the IL-8 chemokine pathway upregulated in CD70+ gliomas by radiation to enhance CNS tropism and persistence in preclinical glioma models. METHODS: The CD70 CAR-1.0 was respectively linked with IL-8 receptors, CXCR1 and CXCR2 and cloned into a retroviral vector. The CAR-transduced T cells were tested against GBM lines (including primary tumors) *in vitro* and human GBM xenograft. The CAR-T cell tumor trafficking/persistence, phenotype, and antitumor efficacy were evaluated. RESULTS: Radiation markedly enhanced the secretion of IL-8 by glioma cells. While mice receiving unmodified CD70 CAR-T cells undergo gradual disease progression with anergic/exhausted phenotypes, the IL-8R-modified CAR-T cells illustrate markedly enhanced tumor migration and persistence and induce complete tumor regression and long-lasting immunologic memory. CONCLUSION: We have co-opted IL-8 release from radiated malignant gliomas, to enhance intra-tumoral T cell trafficking through a new CAR design for maximal anti-tumor activity, thus provide a novel strategy for CAR-T therapy targeting these tumors.

IMMU-57. SEQUENTIAL TWO-RECEPTOR PRIMING CAR SYSTEM TO OVERCOME HETEROGENEOUS ANTIGEN EXPRESSION

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Heterogeneous expression of target antigens can allow tumor escape from chimeric antigen receptor-transduced T-cell (CART) therapy targeting a single antigen. While epidermal growth factor receptor (EGFR)vIII represents a glioblastoma (GBM)-specific antigen, its expression is heterogeneous within the tumor. On the other hand, most other antigens expressed more uniformly in GBMs are non-mutated, glioma-associated antigens (GAAs), such as EphA2. Although these GAAs are not expressed in the normal brain, they are expressed at low levels in other normal organs. As a way to safely target GAAs in the tumor without attacking normal cells expressing the same GAAs outside of the brain, we adapted a novel synthetic Notch (synNotch) receptor system, and established a "prime and kill" sequential two-receptor CAR circuit: the first is a transcriptional CAR against EGFRvIII, and the second is a CAR against a GAA (e.g. EphA2). When the first CAR binds to EGFRvIII, it induces the expression of the second CAR. While the first CAR does not trigger the cytotoxic function itself, the second CAR mediates the cytotoxicity upon recognition of the target GAA. We have validated this system *in vitro* using the mixture of U87 GBM cells and those transduced with EGFRvIII. SynNotch CART effectively lysed both EGFRvIII+ and EGFRvIII-negative U87 cells when they are mixed, but never lysed EGFRvIII-negative cells in the absence of EGFRvIII+ cells. In immunocompromised mice bearing intracranial U87-EGFRvIII+/- tumors, intravenous infusion of synNotch CART resulted in long-term (over 60 days) survival and eradication of the heterogenous tumor in 5 of 8 mice. Furthermore, when mice also bear subcutaneous, EGFRvIII-negative U87 tumors along with the intracranial U87-EGFRvIII+/- tumors, synNotch CART did not affect the subcutaneous tumor, indicating the local but not systemic effects of synNotch CART. These data provide a strong basis for developing synNotch CART therapy for GBM.

IMMU-58. REDUCED NEOANTIGEN EXPRESSION AS A POSSIBLE IMMUNE EVASION MECHANISM DURING GLIOMA PROGRESSION

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Limited success of immune-based therapies in glioma might be in part due to the lack of enough neoantigens (neoAgs) that can be targeted by immune effector cells. In addition, gliomas also show dynamic clonal evolution over time, possibly leading to immunoediting during progression. In this study, we evaluated neoAg expression change and its relation to immune microenvironment using matched primary and recurrent tumor samples from 25 glioma patients [8 IDH-wildtype glioblastoma (GBM), 9 IDH-mutant astrocytoma (LGG-A), and 8 oligodendroglioma (LGG-O)]. Predicted neoAgs (p-neoAgs) deriving from missense mutations were identified by whole-exome sequencing (WES) analysis and the MHC class I binding prediction algorism NetMHCpan2.8. Expressed neoAgs (e-neoAgs) among the p-neoAgs were determined by incorporating RNA sequencing (RNA-seq) data. The ratio of e-neoAgs to p-neoAgs ("neoAg expression ratio") on each sample significantly decreased at recurrence ($p = 0.003$) and was particularly strong in neoAg with a higher affinity for MHC class I. Similar results were obtained for GBM, LGG-A, and LGG-O, when separately analyzed. RNA-seq-based differential gene expression analyses including gene ontology analysis and pair-designed gene set enrichment analysis illustrated that the cases with strongly reduced neoAg expression ratio compared to primary counterpart (top 8), but not those without (bottom 8), retained gene expression related to antigen presentation machinery and gained immune effector cells at recurrence. These *in silico*findings were consistent with immunohistochemistry for CD8 and MHC class I on tumor samples. These data may suggest that the tumor cells with reduced neoAg expression survived at recurrence as a result of persistent anti-tumor immune responses in some gliomas under the standard treatment. Reduced neoAg expression and impaired APM during progression may play complementary roles in the immune evasion of gliomas.

IMMU-59. DETECTION OF HUMAN CYTOMEGALOVIRUS ANTIGENS IN MALIGNANT GLIOMAS AS AN IMMUNO-THERAPEUTIC TARGET

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INTRODUCTION: Findings describing the existence of cytomegalovirus (CMV) antigens in glioblastoma have been controversial since first report in 2002 by Cobbs et al. Since this first publication, there were many studies to confirm the detection of CMV in malignant gliomas using a variety of methods such as immunohistochemistry, PCR, in situ hybridization, and immunofluorescence assay under confocal microscope. For confirming the presence of CMV proteins, we undertook immunofluorescence (IF) staining of CMV pp65 and IE-1 antigens in human primary

malignant gliomas. Also, we investigated CMV specific CTL response on our primary cultured cells using pp65 or IE-1 specific CTLs obtained from healthy donors. RESULTS: First, we analyzed serum CMV immunoglobulin M and G index, and CMV DNA copy numbers using real-time(RT) PCR from 20 malignant glioma patients. All of patients showed positivity of IgG index and negativity of IgM and CMV DNA copy numbers. Second, we stained 15 primary malignant glioma cell lines using pp65 or IE-a specific antibody and the expression of CMV antigens were visualized by confocal fluorescence microscope. We found all positive results (15/15) in primary malignant gliomas, using fibroblast as a negative control and CMV-infected U87 as a positive control. Third, we generated pp65 or IE-1 specific CTLs using mRNA-pulsed dendritic cells from HLA partially matched heathy donors. Using theses CTLs, we revealed positive IFN-gamma ELISPOT assay results. CONCLUSION: We demonstrated not only the presence of CMV proteins (pp65, IE-1) in malignant gliomas but also elucidated that CMV could be an immuno-therapeutic target for adoptive cellular therapy.

IMMU-60. MAPPING TUMORAL AND IMMUNE HETEROGENEITY IN PD-1 RESPONSIVE GLIOBLASTOMA

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Clonal evolution and the immune microenvironment in glioblastoma harbor critical clues to its known but poorly understood treatment resistance. To assess the potential benefit of immune checkpoint blockade (ICB) in glioblastoma it is critical to determine the immune interactions in the tumor microenvironment. We created a tumor-immune interaction map by computing the mutational and neo-epitope tumoral burden, along with the magnitude and clonality of infiltrating lymphocytes, from initial and recurrent tumor of a patient with a glioblastoma *IDH1* wild type, unmethylated O^6-methylguanine–DNA methyltransferase (MGMT) promoter who benefited from ICB. RNA sequencing was performed on samples from the primary and three spatial sectors of the recurrence. Data analyses included intra-tumoral gene expression including MHC-I allele-specific expression, expressed relative mutation, neo-epitope prediction, B/TCR profiling and correlation with analysis of immune cells by multiplex immunohistochemistry (MIHC). We found no evidence of hypermutation in the recurrence, a relative absence of immune infiltration in all recurrences despite the expression of a number of neo-epitope generating mutations (including a novel clonal mutation of EGFR), and compelling evidence of epigenetically driven aberrations in one sector of the recurrence. The differential tumor infiltrating lymphocyte burden observed between recurrent and primary biopsy, indicates immunosuppressive recurrent tumor microenvironment concurred by MIHC data that revealed a relative abundance of B-cell infiltrate. Neo-epitope editing (production) relative to primary is observed in 2/3 (1/3) recurrent tissues, consistent with relative somatic mutation overlap. Immune selection pressure induced from previous radiation/chemotherapy may be implicated in increased tumor heterogeneity and immune editing. Relative extended survival and response to ICB and VEGF-A inhibition can occur in IDH1 wild type, undetected MGMT and absent hypermutated phenotype GBM and warrant further studies to better determine the spectrum of response to PD-1 inhibition. Multi-regional analysis of recurrence may be give clues on failure of ICB.

IMMU-61. INHIBITION OF MUTANT IDH1 WITH AGI-5198 ENHANCES THE EFFICACY OF RADIOTHERAPY ELICITING IMMUNOLOGICAL MEMORY AND IMPROVING OVERALL SURVIVAL

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Mutant IDH1 gliomas have are heterogeneous tumors with a high incidence of relapse. We are studying a glioma subtype, genetically characterized by inactivating mutations in α-thalassemia/mental retardation syndrome X-linked (ATRX) gene, TP53 and gain of function mutations in isocitrate dehyrdogenase 1 (mIDH1). Mutation in IDH1 converts α-ketoglutrate (αKG) to 2-hydroxyglutarate (2HG), an oncometabolite that inhibits histone and DNA demethylases, leading to a hypermethylated tumor phenotype. This leads to epigenetic reprograming of the tumor trancriptome. AGI-5198 a specific mIDH1inhibitor blocks the production of 2HG within the mIDH1 glioma cells. Our *in-vitro* data using AGI-5198 in combination with radiation, which is the standard of care for mIDH1 glioma patients, demonstrated that mIDH1 inhibition confers radiosensitivity to the tumor cells. To determine whether AGI-5198 would be an effective radiosensitizer *in-vivo*, we used a mIDH1 glioma transplantable mouse model. Animals were implanted with mIDH1 tumor cells and at day seven post tumor implantation; they were treated with radiation followed by administration of AGI-5198. With systemic delivery of AGI-5198, we observed 40% long term survivors(>90 days) in the single modality treatment. Our results also demonstrate that the combination of AGI-5198 and radiation therapy significantly prolongs the median survival (MS) of mIDH1 glioma bearing mice (~1.5 fold vs controls); eliciting strong antitumor activity, eradicating 40% of the established mIDH1 gliomas. In addition, when the long term survivors were rechallenged with mIDH1 tumor cells in the contralateral hemisphere, without further treatment, all the mice remained tumor free, indicating the development of anti-glioma immunological memory. Collectively, these findings support the clinical testing of AGI-5198 as an adjuvant therapy for patients with mIDH1 glioma.

IMMU-62. LOW-GRADE GLIOMA EXCLUDE CD8 T CELLS, WHICH IS ACCOMPANIED BY LOW EXPRESSION OF CHEMO-ATTRACTANTS, NOT IMMUNOGENIC ANTIGENS

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In multiple tumor types, prediction of response to immune therapies relate to the presence, distribution and activation state of tumor infiltrating lymphocytes (TILs). Little is known, however, about the immune contexture of TILs in gliomas. To this end, we assessed whether low and high-grade glioma (LGG and HGG) differ with respect to number, location and tumor reactivity of TILs; as well as expression of molecules involved in the trafficking and activation of T cells. TILs were collected from glioma samples and CD3 and CD8 T cells were quantified by flow cytometry (n=10) and immunofluorescence (n=35). Neo-antigen load and expression of Cancer Germline Antigens (CGAs) were assessed using whole exome sequencing and RNA-seq. TIL-derived DNA was sequenced and the variable domain of the TCRβ chain was classified according to IMGT nomenclature (n=5). QPCR was used to determine expression of T cell-related genes (n=20). T cell numbers were significantly lower in LGG. In HGG, CD8 T cells migrated into tumor tissue, whereas in LGG, they mainly remained in close vicinity to blood vessels. Although HGG had significantly more genetic changes, the number of neo-epitopes was only 2 in both tumor types. We also did not observe a difference in the expression of CGAs nor in dominant TCR-Vb gene usage. The low number and perivascular location of CD8 T cells in LGG was accompanied by a low expression of chemo-attractants CXCL9 and CXCL10, adhesion molecule ICAM1 and CD8 T cell effector molecule GZMK. LGG have lower numbers of TILs compared to HGG, potentially linked to decreased T cell extravasation and activation. We have found no evidence for distinct tumor reactivity of T cells in either tumor type. Our data argue that LGG would require sensitization to enhance numbers and trafficking of CD8 T cells prior to immune therapies, such as checkpoint inhibitors.

IMMU-63. IDH1 MUTATION REGULATE MYELOID CELLS PLASTICITY MEDIATING ANTI-GLIOMA IMMUNOTHERAPY

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Molecular characterization studies have illustrated that mutation in isocitrate dehydrogenase (mIDH1) enzyme, which is present in most patients with low grade glioma (LGG) and secondary glioma is correlated with better prognosis and survival [1]. We hypothesize that mIDH1 impacts tumor immunity by altering the phenotype and function of tumor infiltrating immune cells. To examine the role of mIDH1 on the immune tumor microenvironment (TME), we generated LGG glioma models using sleeping beauty system [2]. Mice bearing mIDH1 tumors exhibited longer median survival (MS) compared to the tumors harboring wtIDH1 (MS=163 vs. MS=70-day post-injection). Transcriptome analyses showed a positive enrichment in immune-stimulatory related gene families in mIDH1 tumors vs. wtIDH1 tumors; consistent with Human RNA-seq analysis from TCGA analysis. Using co-culture experiments followed by the in vivo characterization of immune cells within the TME, we showed that mIDH1 have higher

expansion of CD11b+ Gr-1+ myeloid cells compared to wtIDH1 tumors (%MDSC/CD45+: 67.0% vs. 45.06%; P<0.05). Interestingly, CD11b+ Gr-1+ myeloid cells (MDSCs) from mIDH1 tumor expressed lower levels of immunosuppressive markers such as PD-L1 and CD80 which are involved in T-cell checkpoint inhibition. Consistent with this, TME-derived CD11b+ Gr-1+ myeloid cells from mIDH1 tumors didn't inhibit tumor antigen specific T-cells' expansion. Further analysis indicated that mIDH1 MDSCs also express lower levels of maturation markers suggesting that MDSCs from mIDH1 are at earlier maturation/differentiation stage. Interestingly, MDSCs depletion did not improve efficacy of immunotherapy in mIDH1; whilst in wtIDH1 bearing animals, survival increased to 70% when MDSCs depletion was combined with immunotherapy. In conclusion, mIDH1 expression in glioma cells impact the myeloid cells' compartment possibly by changing the cytokine's repertoire secreted by the tumor cells. This leads to reprogramming of myeloid cells within the TME which exhibit a non-immunosuppressive phenotype. 1. Ceccarelli, M., et al., 2016 2. Koschmann, C, et al., 2016

IMMU-64. A DECADE OF RESEARCH TARGETING IMMUNOSUPPRESSIVE IDO1 IN GLIOBLASTOMA: NEARING THE FINISH LINE OR JUST BEGINNING THE MARATHON?

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INTRODUCTION: The Phase III ECHO-301/KEYNOTE-252 trial results showing that, the combination of PD-1 inhibitor (Keytruda), combined with the IDO1 enzyme inhibitor (epacadostat), fails to improve PFS, as compared to single-agent treatment with PD-1 inhibitor, alone, has led to a cascade of IDO1-inclusive clinical trial discontinuations; and raised critical questions for whether IDO1 inhibition is important to patients with cancer. The first *in vivo* report demonstrating an ability for IDO1 to suppress the immune response, was shown in a syngeneic mouse mastocytoma model (Uyttenhove *et al*., 2003; Nature Med.). The group demonstrated that, tumor cell IDO1 expression led to the suppression of cancer immunity. High expression for IDO1 was also detected in ~90% of human patient-resected glioblastoma (GBM) specimens. In 2012, I led a report showing that, wild-type C57BL/6 mice intracranially-injected with syngeneic GL261, and knocked down for IDO1 expression (GL261-IDO1kd), led to the spontaneous rejection of brain tumors (Wainwright *et al*., 2012; CCR). Strikingly, the same GL261-IDO1kd tumor grew out normally in mice deficient for $CD4^+$ and/or $CD8^+$ T cells, collectively confirming the immunosuppressive role of glioma cell IDO1. In 2017, my group demonstrated a number of novel observations that began questioning whether IDO1 enzyme activity suppresses the immune response, or rather, whether enzyme activity serves a proxy for an alternative, uncharacterized immunosuppressive mechanism (Zhai *et al*., 2017a; CCR, Zhai *et al*., 2017b; BBI). In 2018, we unexpectedly discovered that, IDO1 becomes targetable in non-tumor cells after treatment with ionizing radiation and PD-1 mAb. Strikingly, immunosuppression mediated by tumor cell IDO1, was unaffected by a pharmacologic IDO1 enzyme inhibitor. CONCLUSIONS: These cumulative data have prompted us to create novel transgenic models that will allow us to further understand how to effectively target IDO1 in glioblastoma, and potentially, to serve as valuable tools for the validation of second generation IDO1 inhibitors.

IMMU-65. IL-6 DRIVES ALTERNATIVE MACROPHAGE ACTIVATION AND IMMUNE SUPPRESSION IN GLIOBLASTOMA THROUGH PPARγ/HIF-2α

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Glioblastoma (GBM) is the most common and most aggressive primary brain tumor in humans. Immunotherapy holds great promise for GBM treatment. However, current immunotherapy of solid tumors, primarily by targeting tumor-associated T cells, remains a big challenge, largely due to limited T cell infiltration and activation in the immunosuppressive microenvironment. Here our work reveals a macrophage-dependent mechanism for tumor immunosuppression, by which IL-6 induces alternative macrophage activation in GBM microenvironment. We show that IL-6 induces robust arginase-1 expression and macrophage alternative activation, mediated through Akt/mTOR/peroxisome proliferator-activated receptor (PPAR)-γ-dependent transcriptional activation of hypoxia-inducible factor (HIF)-2α. Furthermore, genetic ablation of IL-6 abrogates macrophage alternative activation, inhibits tumor growth and progression, enhances tumor-associated T cells' activity, and improves animal survival in a genetically engineered mouse GBM model. Notably, administration of anti-IL6 neutralizing antibody remarkedly reverses tumor immune suppression and promotes infiltration of T cells and neutrophils, leading to inhibited tumor growth and improved animal survival in the GBM-bearing mice. Finally, analysis of TCGA database suggests that high expression of IL-6 correlates with poor overall survival in human patients with low-grade glioma and GBM. Taken together, our findings suggest that IL-6 blockade may offer exciting opportunities for reversing immunosuppression in GBM, serving as a stand-alone therapeutic strategy or a combined treatment with T cell-based immunotherapy.

IMMU-66. A NOVEL METABOLITE TO INCREASE EFFICACY OF CELLULAR IMMUNOTHERAPY IN THE TREATMENT OF GLIOBLASTOMA

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INTRODUCTION: Immune cell migration is critical for cellular immunotherapy efficacy in the treatment of glioblastoma (GBM). Sarcosine is a non-toxic metabolite that is associated with a migratory phenotype in prostate cancer cells. We utilized sarcosine to increase migration of dendritic cells (DC) and T cells to increase efficacy of cellular immunotherapy. HYPOTHESIS: Sarcosine increases cellular migration and will improve tumor outcomes when combined with cellular immunotherapy platforms in the treatment of GBM. METHODS: DC and T cell migration was evaluated in vitro using transwell plates and in vivo using flowcytometry and immunofluorescence microscopy. The impact of sarcosine loaded cells on tumor outcomes was measured by testing a DC vaccine platform in the treatment of murine tumor models. Genomic expression of cytokines on sarcosine treated cells was tested using RT-PCR. RESULTS: Cells were efficiently loaded with sarcosine by simply adding sarcosine to the culture media (20mM). DCs and T cells loaded with sarcosine demonstrated increased migration in vitro ($p<0.0001$). Mice treated with DC vaccination in the setting of sarcosine demonstrated significantly increased DC migration to draining lymph nodes and spleens ($p<0.05$). Gene expression analysis demonstrated that sarcosine caused upregulation of cytokines (IFN g,Xcl1,Fasl,Csf2,CCL19,Bmp2,IFNa2 and IL27) and downregulation of other cytokines (Ccl22,IL1b,Cxcl3,Ccl5,IL9 and IL18)($p<0.05$). Vaccination with sarcosine loaded OVA-DCs led to increased proliferation of antigen specific T cells compared to regular OVA-DC vaccination ($p<0.05$). B16F10-OVA tumor bearing animals treated with sarcosine loaded OVA-DC vaccines had significantly reduced tumor growth and prolonged survival compared to animals treated with OVA-DC vaccines without sarcosine ($p<0.0001$). CONCLUSION: Sarcosine loaded DC vaccines resulted in suppressed tumor growth and improved survival in a murine model. These effects are partially mediated by changes in cytokine expression in sarcosine loaded DCs. Further experiments testing sarcosine loaded T cells and the mechanism of increased migration in sarcosine loaded immune cells are underway.

IMMU-67. PERSONALIZED TUMOR MRNA LOADED LIPID NANOPARTICLES PRIME THE SYSTEMIC AND INTRATUMORAL GBM MILIEU FOR RESPONSE TO IMMUNOTHERAPY

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BACKGROUND: In a randomized phase III study for recurrent glioblastoma (GBM), combination therapy with immune checkpoint inhibitors (ICIs) nivolumab and ipilimumab failed to improve overall survival. Since ICIs appear to be dependent on host-myeloid (and not tumor-cell) expression of PD-L1 (Tang et al. JCI, 2018), we sought to assess if tumor-mRNA nanoparticles (NPs) could prime the systemic and intratumoral GBM milieu with activated PD-L1+ host-myeloid cells sensitizing immunologically 'cold' tumors to immunotherapy. METHODS: We systemically administered personalized tumor-derived mRNA encapsulated in translatable lipid-NPs with excess positive charge for enhanced delivery and transfection of peripheral/intratumoral antigen presenting cells (APCs) in murine and large animal canine malignant glioma models. RESULTS: RNA-NPs activate systemic immunity within twenty-four hours, inducing significant increases in the percentage of CD11c+ myeloid-cells expressing PD-L1 and CD86. After only a single RNA-NP vaccine, the bulk of APCs within the spleen, liver, lymph nodes, bone marrow and tumor display an activated phenotype. These PD-L1+ APCs (CD11c+MHC-II+CD86+PD-L1+ cells from intracranial tumors) did not

suppress immunity, but rather, heightened interferon-(IFN)-γ reactivity. Addition of ICIs (to animals primed with RNA-NPs) augmented peripheral/intratumoral PD-1+CD8+ cells and mediated synergistic anti-tumor efficacy in settings where ICIs alone did not confer therapeutic benefit. These synergistic effects were mediated by type–I-interferon released from PD-L1+ plasmacytoid dendritic-cells. In translational studies, personalized mRNA-NPs (from whole tumor-transcriptome) were safe and active in a client-owned canine with a spontaneous malignant glioma. In this patient, RNA-NPs elicited robust immunologic activity with increased percentages of activated PD-L1+ APCs and IFNγ+CD8+ cells. CONCLUSION: RNA-NPs elicit widespread immune activation concomitant with inducible PD-L1 expression on intratumoral APCs that can be therapeutically exploited. Since RNA-NPs bypass cost/complexity of cellular therapeutics, are amenable to central distribution, and can be made within days of tumor resection, these formulations can be expeditiously translated as biomodulators of GBM immunogenicity.

IMMU-68. SINGLE-CELL PROTEOMIC ANALYSIS OF IMMUNE CELL RESPONSE TO CHECKPOINT BLOCKADE USING 30-PARAMETER FLOW CYTOMETRY

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BACKGROUND: Biomarkers of response to immunotherapy are critical to evaluate efficacy and ultimately select patients most likely to benefit from this potentially toxic treatment. Peripheral blood mononuclear cells (PBMC) are easily collected and offer abundant lymphocytes, yet the molecular determinants of cells relevant to a therapeutic response have not been determined. To identify such cells, likely rare, we developed four state-of-the-art flow cytometry assays that quantify 65 unique proteins on single-cells for comprehensive, high-throughput proteomic interrogation of PBMC and tumor-infiltrating lymphocytes. Our goal is to identify biomarkers and predictors of response to immunotherapy as well as to find potential peripheral correlates of immune responses to the tumor itself. METHODS: Molecular determinants of B, NK, DC, classical T, invariant T, NKT, and MAIT immune cell lineages and differentiation stages, as well as functional markers including cytokines, cytokine receptors, activation/senescence markers, proliferation, and markers of cytotoxicity were chosen as potential biomarkers of response. Proteomic analyses were performed on PBMC from healthy donors and patients with GBM who received checkpoint blockade therapy. RESULTS: Changes in cellular composition, cell differentiation and functional status were quantified in this comprehensive analysis of healthy donor and patient PBMC. Cellular expression patterns varied by cell activation and differentiation status; highlighting the importance of detailed subset analyses. Potential biomarkers were selected using linear discriminant analysis to identify proteomic differences between healthy donor and patient, as well as longitudinal patient PBMC samples. CONCLUSIONS: Using cutting-edge flow cytometry, we generated a comprehensive analysis of molecular and cellular changes that occur in PBMC in response to immune checkpoint blockade. Additionally, we identify sources of interpatient variation that may obscure subtle proteomic changes. This technological advance provides high-throughput proteomic analysis of live lymphocytes, which can then be sorted for downstream molecular analyses: a critical step in biomarker validation and discovery.

IMMU-69. PROGNOSTIC IMPLICATION OF B-CELL INFILTRATE IN PATIENTS WITH GLIOBLASTOMA (GBM)

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BACKGROUND: One of the purported reasons of ineffectiveness of immunotherapy for GBM is the meager immune response in the tumor microenvironment. We have observed that GBM can harbor dense B-cell infiltrate, but their prevalence and function is unknown. Pre-clinical studies have shown that B-cells can act as antigen-presenting cells which can stimulate T-cell proliferation; however, B-cells can also downregulate effector T-cells by secreting immunosuppressive cytokines including IL-10. Our study aims to determine the roles of B-cells in GBM and their association with patient outcomes. METHODS: Multiplexed immunohistochemistry, imaging and quantitation was performed on primary GBM samples obtained between 1/2008-8/2015. Intratumoral $CD20^+$B-cells and $CD8^+$T-cells infiltrate were enumerated from 3 serial GBM sections. Relevant prognostic clinical and molecular variables were reviewed. We also analyzed the association of MS4A1 gene encoding B-cell antigen CD20 with survival from the TCGA. RESULTS: We observed an average infiltrate of 5.5 $CD20^+$ cells/$mm^2$and 36.7 $CD8^+$cells/mm^2tumor. B-cells were stratified into $CD20^{low}$(12 patients) and $CD20^{high}$groups (36 patients) with 0.24 B-cells/mm^2 cut off. Kaplan-Meier analyses indicated that the $CD20^{low}$-group had better overall survival (OS) than the $CD20^{high}$cohort (31.7 versus 18.7 months; p=0.02), while a trend toward improved survival was observed with high $CD8^+$T-cell infiltration (p=0.06). Cox proportional hazard demonstrated an association between decreased OS and $CD20^{high}$cohort (HR=2.8, p=0.03), but no significance seen between $CD8^+$infiltration and OS (p=0.16). A positive correlation was detected between $CD8^+$and $CD20^+$density (p CONCLUSIONS: Our results suggest that B-cell infiltrate carries an inverse correlation with survival. We hypothesize that $CD20^+$B-cells may be regulatory B-cells, limiting immune activation in GBM. Further studies are underway to understand the role of B-cells in the GBM microenvironment.

IMMU-70. GLOBAL IMMUNE FINGERPRINTING IN GLIOBLASTOMA REVEALS IMMUNE-SUPPRESSION SIGNATURES ASSOCIATED WITH PROGNOSIS

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Therapeutic resistance in glioblastoma (GBM) is linked to cancer stem cells and signaling pathway alterations, but the role of local and systemic immune suppression during disease progression is less understood. To determine how the immune system as a whole is altered in GBM patients, a CyTOF panel of 25 immune markers was developed for immune fingerprinting of peripheral blood mononuclear cells (PBMCs). CyTOF analysis was performed on select patients with either favorable or poor prognosis from a cohort of newly diagnosed GBM patients, from whom PBMCs were collected throughout disease progression. Multi-dimensional cluster analysis identified a reduction of Monocytic Myeloid-Derived Suppressor Cells (M-MDSCs) in the patient with favorable prognosis, while the patient with poor prognosis had higher levels of M-MDSCs. Additionally, when LGG patients were compared to GBM patients, LGG patients had more Dendritic cells and Natural Killer cells as compared to GBM patients. To understand whether this elevation in MDSC was unique to GBM or whether MDSCs were high across primary and secondary brain tumors, a cohort of 259 patient PBMC samples was analyzed. GBM patients had a significant elevation in MDSCs in blood, but not immunosuppressive T regulatory cells as compared to non-malignant brain tumors. To determine whether MDSCs in the GBM microenvironment also correlate with survival, a cohort of patients with matched primary and secondary resections were analyzed for MDSCs by immunofluorescence, which determined that intra-tumoral MDSCs correlate with poor prognosis in recurrent samples, while general myeloid infiltration correlated with a good prognosis. These studies identify low MDSC immune signatures that are linked to enhanced pro-inflammatory cells and overlap between GBM patients with a good prognosis and LGG patients, promoting the idea of targeting MDSCs in GBM patients to alter their immune status to that of a lower grade tumor.

IMMU-71. EVALUATING THE COMPATIBILITY OF TUMOR TREATING ELECTRIC FIELDS WITH KEY ANTI-TUMORAL T CELL FUNCTIONS

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BACKGROUND: Combining Tumor Treating electrical Fields (TTFields) with immunotherapy is a rational approach due to their different mecha-

nisms of action (MOA) and to TTFields' ability to induce immunogenic cell death (ICD). Conversely, TTFields may interfere with immune functions critical for effective T cell responses. METHODS: T cells from healthy donors' peripheral blood or from viably dissociated glioblastoma samples were cultured under normal or TTFields conditions, with or without superantigen-stimulation. Eight-color flow cytometry was used to assess T cell responses by monitoring select pivotal antitumoral functions: proliferation (CFSE), IFNγ secretion, cytotoxic degranulation (CD107a), activation/exhaustion (PD1) and viability. Direct cytotoxicity was evaluated using chimeric antigen receptor (CAR) T cells. RESULTS: The viability of stimulated T cells that attempted to proliferate decreased under TTFields, in line with TTFields' MOA. Small or no reductions in viability were found in activated T cells that did not attempt to proliferate and in unstimulated T cells. The functionality of stimulated peripheral-blood T cells and tumor-infiltrating T cells (TILs) under TTFields was unhindered: T cells exhibited comparable PD1 upregulation, IFNγ secretion and CD107a expression as controls. T cell polyfunctionality, associated with effective antitumoral responses, was retained under TTFields conditions. PD1-expressing TILs, a subset containing most of the tumor antigen-specific TILs, exhibited unaltered viability and functionality under TTFields. CAR T-cells, which utilize the same killing machinery as unmodified T cells, exhibited unaltered cytotoxic capability under TTFields. Gene expression analysis of GBM tissues obtained before and after patients' treatment with chemoradiation or chemoradiation+TTFields, demonstrated, in TTFields treated patients, increases of transcripts associated with antitumoral responses (CD8, NKp46, GranzymeB, Perforin), and decreases in protumoral-associated myeloid-compartment transcripts (CD66b, CD163, HLADR) CONCLUSIONS: All antitumoral T cell functions examined, with the exception of proliferation, were unhindered by TTFields. Our findings warrant the further preclinical and clinical investigation into the combination of TTFields and immunotherapy.

IMMU-72. TARGETING GLIOBLASTOMA STEM CELLS USING A SECOND-GENERATION EGFRvIII SPECIFIC PEPTIDE VACCINE STRATEGY

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A large proportion of GBM tumors express an altered, constitutively active version of the EGF receptor referred to as EGFRvIII. Along with increasing proliferation and inhibiting apoptosis, expression of EGFRvIII is also a GBM stem cell marker. Targeting tumor stem cells based on EGFRvIII expression may therefore improve survival for GBM patients. As such, several clinical trials using a peptide vaccine directed against EGFRvIII have shown promising results. We have explored improved versions of our original EGFRvIII peptide vaccine and have identified a candidate that shows a 60% improvement in murine subcutaneous tumor models. In this study, we further assessed the efficacy of this 2nd generation vaccine in a murine GL261 intracranial tumor model. Mice that received the EGFRvIII peptide vaccine survived 40% longer (median) than non-vaccinated mice. This response required both CD4+ and CD8+ T cells as the observed survival benefit was lost when either of these cell populations was depleted. The specificity of this vaccine strategy was valicated by an *in vitro* cytotoxicity assay which demonstrated that T cells derived from EGFRvIII peptide vaccinated mice were capable of specifically killing EGFRvIII expressing GL261 target cells *in vitro,* while demonstrating no cytotoxitiy against EGFRvIII negative cells. Treatment with the peptide vaccine increased the abundance of tumor infiltrating CD8+ T cells (p=0.0017) and altered the intra-tumoral CD4+ to CD8+ cell ratio (p=0.0015). Moreover, relative to control mice, tumor infiltrating CD8+ T cells in vaccinated mice show significant expression of the conventional immune checkpoint receptor PD-1, indicating that combinational therapies may potentiate the vaccine induced survival benefit in this model. We are currently assessing the utility of both adjuvant and immunomodulatory combinations in an attempt to further increase the efficacy of this second generation peptide vaccine.

IMMU-73. GLIOMA IN SITU VACCINATION WITH COWPEA MOSAIC VIRUS NANOPARTICLES

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Glioblastoma multiforme is the most malignant brain cancer affecting adults. Despite recent advancements, the current prognosis is dismal with median survival of 12 months post diagnosis even with multi-modal therapy. Cancer immunotherapy has made significant advances in treatment of multiple cancer types and is a promising avenue for development for glioma treatment. However, the highly immunosuppressive tumor microenvironment remains a challenge. The tumor microenvironment induces tolerance, exhaustion or death of T effector cells, protecting the tumor from most immune effector cells. Here we have utilized a 30 nm-sized virus like particle (VLP) derived from the plant virus Cowpea mosaic virus (CPMV) as an immunotherapy. The VLP is applied intratumorally as an in situ vaccine, modulating the tumor microenvironment and stimulating anti-tumor immunity. The VLPs are ingested by innate immune cells driving a cascade of immunological events, which leads to activation of the adaptive immune system and cancer cell death. We have demonstrated that weekly VLP treatment of immunocompetent mice with a high tumor burden of GL261 cells creates a localized inflammatory response that kills tumor cells. MRI imaging demonstrates GL261 tumor bearing mice have increased edema and swelling indicative of immune infiltration after treatment. Histological analysis with markers for immune cells support this finding. The ensuing immune response was also characterized using flow cytometry analysis on brain and spleen tissues and using ELISAs to determine cytokines milieu following the treatment.

INNOVATIONS IN PATIENT CARE

INNV-01. PROTECT STUDY: PROPHYLACTIC SKIN TOXICITY THERAPY WITH CLINDAMYCIN AND CLOBETASOL OR SKIN BARRIER IN GLIOBLASTOMA PATIENTS TREATED WITH TUMOR TREATING FIELDS

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BACKGROUND: Tumor treating fields (TTFields) are non-invasive, antimitotic low intensity, intermediate frequency (200 kHz) alternating electric fields. TTFields have demonstrated survival advantage when combined with maintenance TMZ in newly diagnosed glioblastoma. TTFields are applied continuously (>18 hours/day) using a set of four transducer arrays placed on the patients scalp. The main TTFields-related adverse event is mild-moderate dermatitis and pruritus. Strategies that mitigate skin events may improve quality of life, adherence, and prevent treatment interruptions. Several clinical trials are evaluating the concomitant use of TTFields with a variety of immunotherapies which are also associated with dermatologic toxicities. This multicenter, prospective, randomized, double-blind pilot study will test the efficacy and safety of topical clobetasol and clindamycin and a skin barrier to prevent skin events from TTFields. METHODS: Patients (n=90) will be randomized to receive either: clobetasol propionate, and clindamycin (n=30), topical skin barrier (n=30), or 70% ethanol (n=30, as control group). The primary study outcome is the difference in grade 2 device-related skin toxicity between the arms at study completion (day 90 ± 14 days). The subjects will be assessed (clinical, QoL, interventions) on day 0, 30, 60, at the end of study day 90, grading will be conducted by CTCAE v5.0 and blinded photograph evaluation. A sample size of 90 patients will allow 80% power to detect a 30% difference in grade 2 dermatitis between the treated and non-treated sides with a two-side alpha type I error rate of 5%. Estimated time of accrual is 36 months with an accrual rate of 2–3 patients per month. Anticipated time to complete the study is 39 months.

INNV-03. SAFETY AND ADVERSE EVENT PROFILE OF TUMOR TREATING FIELDS USE IN THE EMEA REGION A REAL-WORLD DATA ANALYSIS

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INTRODUCTION: Tumor Treating Fields (TTFields) is approved for the treatment of newly diagnosed and recurrent glioblastoma. The efficacy and safety of TTFields in newly diagnosed GBM was previously demonstrated in the EF-14 phase 3 trial (n=695). Optune + temozolomide significantly improved survival outcomes compared to temozolomide alone (median OS: 20.9 vs 16.0 months, p METHODS: A review of global post-market surveillance data including patients treated with Optune in the EMEA region. Adverse events reported to the Novocure safety department were analyzed based on the MedDRA body system (system organ class) preferred terms. RESULTS: A total of 8025 patients were treated with Optune globally (newly diagnosed glioblastoma 46.1%, recurrent glioblastoma 46.2%, other 7.7%). Of these, 1496 patients were treated in the EMEA region (Israel, Switzerland, Germany and other European countries). In this cohort, as expected, skin reaction was the most prevalent adverse event affecting 25% of patients. Other adverse events included: General decline in health (24%), Nervous system disorders (18%), Injury at any location (5%), Gastrointestinal disorders (4%), and Musculoskeletal disorders (3%). CONCLUSION: This retrospective analysis demonstrates the safety profile of Optune in an EMEA patient cohort. The most common adverse event registered was skin reaction, which was also found in the phase 3 EF-11 trial for recurrent GBM and in the EF-14 trial for newly diagnosed GBM patients. No other device-related adverse events were reported. In summary, these results emphasize the safety of Optune in GBM treatment and its consequent adoption in the EMEA region.

INNV-04. SAFETY AND ADVERSE EVENT PROFILE OF TUMOR TREATING FIELDS IN GLIOBLASTOMA A GLOBAL POST-MARKET SURVEILLANCE ANALYSIS

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INTRODUCTION: The efficacy of Tumor Treating Fields (TTFields) in newly diagnosed glioblastoma (ndGBM) and recurrent GBM (rGBM) was demonstrated in the EF-14 and EF-11 phase 3 trials. TTFields are administered through transducer arrays placed on the shaved scalp. Skin irritations below arrays were the main adverse event observed with TTFields. We report global post-marketing surveillance data from ndGBM and rGBM patients treated with TTFields. METHODS: Surveillance data were analyzed based on the MedDRA body system (system organ class) preferred terms. RESULTS: A total of 7408 patients were treated with TTFields (ndGBM (n=3697), rGBM (n=3711). Age at the time of TTFields treatment: ndGBM (8 to 91 years) and rGBM (7 – 95 years). Proportion of patients reporting ≥ 1 adverse event was 56% in ndGBM and 36% in rGBM. Skin reaction was the most prevalent adverse event and occurred in 35% and 20% of ndGBM and rGBM patients. The remaining adverse events were fairly evenly distributed between both ndGBM and rGBM patient groups: electric sensation (11% and 6%); heat sensation (10%, 6%); seizures (10%, 8%) and General Physical Health Deterioration (3%, 4%). CONCLUSIONS: This retrospective analysis demonstrates real-life evidence on TTFields use in GBM. The most common adverse event registered was skin reaction, which was also found in the phase 3 EF-11 trial for recurrent GBM and in the EF-14 trial for newly diagnosed GBM patients. The low rate of General Physical Health Deterioration is inline with Health Related Quality of Life (HRQoL) assessments in the Phase 3 GBM studies. These results from post-marketing surveillance support the safety profile of TTFields in the treatment of ndGBM and rGBM.

INNV-06. FACTORS INFLUENCING RECEPTIVITY TO CLINICAL RESEARCH IN AN URBAN NEURO-ONCOLOGY PRACTICE

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Fewer than 3% of cancer patients participate in clinical trials. Nervous system involvement creates additional challenges to accrual including reduced capacity for informed decision-making and increased reliance on caregivers. To better identify barriers to enrollment, we designed and tested surveys to elicit patient perceptions about research and collect data on potential factors influencing receptivity to involvement. A 20-item survey, later expanded to 44, was provided to patients at the time of their first outpatient visit. The survey was voluntary and administered as part of a QI initiative conducted by Columbia's Division of Neuro-oncology over the course of 54 clinical practice days. Questions assessed demographics (race/ethnicity, education, language, employment, marital status); social support; travel burden; medical information and decision-making preferences; receptivity to participation in research and rationales for refusal. Approximately 200 patients received the questionnaire; 139 (70%) responded. Median age was 55 (range: 17–85). There were 70 (50%) men; 55 (40%) self-identified as non-Hispanic White; 20 (14%) were non-fluent in English. Diagnoses included high grade glioma (29%), CNS metastases (24%), meningioma (13%), primary CNS lymphoma (3%) and low-grade glioma (5%). Of 121 responders, 37 (31%) were unwilling to participate in research *a priori*. The percentage increased to 42% if randomization was involved, 61% if placebo-controlled. Logistic regression was performed to assess the impact of factors on respondents' willingness to participate in clinical research. Disease severity was the strongest predictor of receptivity (OR: 3.4; CI 1.3–8.8), but full-time employment (OR: 3.2; CI 1.2–8.5) and the presence of caregiver support (OR: 5.4; CI: 1.2–24.4) were also significant. While further research is needed to better understand barriers to trial enrollment, caregiver influence remains largely unexplored. To that end, Columbia is undertaking a pilot to assess the short and long-term impacts of high grade glioma on caregivers and their role in clinical decision making.

INNV-07. BRACHYTHERAPY IN GLIOBLASTOMA: A SURVEILLANCE, EPIDEMIOLOGY, AND END RESULTS (SEER) ANALYSIS

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There are several justifications for brachytherapy as treatment for glioblastoma, including continuous dose rate, high cumulative dose, and rapid radiation delivery. However, two landmark randomized clinical trials (RCT) have failed to demonstrate clinical efficacy. With the development of novel radiation sources and an improved technology, there has been a resurgence in interest in brachytherapy. Here, we used a large national cancer registry to assess the survival benefit of adjuvant brachytherapy in glioblastoma. We identified patients diagnosed with glioblastoma in the Surveillance, Epidemiology, and End Results (SEER) database between 1975–2015. We grouped patients based on receipt of brachytherapy. We collected information on clinical and demographic variables associated with survival. We analyzed group differences using Student's t-test and Pearson's chi-squared test and studied survival using Kaplan-Meier curves and Cox proportional hazards models. Of 54,589 glioblastoma patients, 325 were treated with adjuvant brachytherapy. Brachytherapy patients were younger, had smaller tumors, and were more likely to receive extensive surgery and chemotherapy. Median survival was 16 months for patients who received brachytherapy compared to 9 months for patients who did not (log-rank $p<0.001$). In univariable analysis, variables associated with improved survival include: younger age, tumors <4 cm, no midline extension, extensive surgery, and treatment with chemotherapy. In a multivariable analysis that controlled for these variables, the association between improved survival and brachytherapy remain robust. In this analysis, the hazard of death was reduced in patients receiving brachytherapy (HR 0.758, $p<0.001$) relative to patients who did not receive brachytherapy. Given the effect size observed, we estimate that previous RCTs did not have sufficient sample size. Our SEER analysis suggests potential efficacy of brachytherapy as a glioblastoma therapy. Interpretation of our analysis need to be framed in the context of the inherent limitations of the SEER database, the two previously published randomized controlled studies, and the available literature.

INNV-08. THE UTILIZATION OF INTRAOPERATIVE CONFOCAL LASER ENDOMICROSCOPY DURING THE FLUORESCENCE GUIDED SURGERY FOR BRAIN TUMORS

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Confocal laser endomicroscopy (CLE) allows intraoperative "optical biopsy" at a cellular level without tissue processing. We report the evolution of this technology and present analysis of recent use of the updated CLE tool on patients during fluorescein sodium (FNa) guided brain tumor surgeries. Our clinical experience with CLE includes 237 patients with gliomas, meningiomas and other CNS pathologies examined ex vivo and in vivo using a Generation1 CLE and 48 patients using a Generation2 CLE (19 HGG, 3 LGG, 11 pituitary adenomas, 2 craniopharyngiomas, 2 metastases, 2 schwannomas, 4 meningiomas, 2 treatment effects, 1 focal cortical dysplasia, 2 hemangioblastomas) examined ex vivo. Acriflavine (AF) and acridine orange (AO) were used ex vivo on selected tissue samples. In vivo CLE during FNa-guided surgery produced 77.7 ± 46.2 (average) images/optical biopsy location. A first diagnostic image was identified within seconds of CLE application. In vivo CLE specificity/sensitivity (FNa) was equal or better than frozen section (94%/91% gliomas, 93%/97% meningiomas respectively). Generation2 CLE showed improved image resolution and system operation for detectable tumor signal with Z-stack 3D imaging compared to Generation1. FNa 2 mg/kg administered during induction of anesthesia was sufficient for wide field tumor fluorescence visualization using the operative microscope Yellow560 mode. However, additional injection of FNa (2–5 mg/kg) before optical biopsy was necessary to provide sufficient CLE image contrast for immediate ex vivo CLE imaging in most of the cases. CLE imaging after rapid ex vivo application of AF and AO revealed more intense and specific contrasted intracellular structural patterns, such as nuclei. Overall, CLE rapidly provided information on tissue architecture and atypical cellular features and has potential to improve the surgery-pathology workflow. Additional injection of FNa during fluorescence-guidance surgery may be necessary for CLE optical biopsy, which may interfere with the operative microscope wide field fluorescence visualization, and require further investigation.

INNV-09. PILOT STUDY OF A SMARTPHONE-BASED SYMPTOM ASSESSMENT (OURBRAINBANK) FOR SUBJECTS WITH GLIOBLASTOMAS

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Self-reports from patients done monthly or every few months in the doctor's office have several limitations, including poor recall, under- or over-

reporting of events, among other biases. We developed a glioblastoma specific app (OurBrainBank) using a platform designed by uMotif, which was previously used in other conditions but has been customized for glioblastoma. All data are sent to a HIPPA compliant database. The subject can view or export their own data as well. Inclusion criteria included age 18 years or older, diagnosis of glioblastoma, English-speaking subject, and availability of a smartphone or tablet. After electronic informed consent, patients completed baseline questionnaires about their treatment and validated surveys (EORTC-QLQ, EORTC-BN20). Certain parameters such as sleep quality, exercise, mood, and fatigue were captured for all patients. In addition, patients picked 6 additional symptoms most relevant to their clinical condition. Over a 2-month period, since the study was IRB-approved and the app made available free of charge on app stores, there have been 591 individual patients downloads (167 on Android and 424 on iOS). Recruitment has relied heavily on social media and patient run online support groups. We will present the data on compliance, patient characteristics and symptoms tracking at the meeting. At this initial stage, OurBrainBank's focus is on capturing patients' symptoms. In the next steps, we plan to collect passive data from smartphones and other device trackers. Additionally, OurBrainBank will enable patients to donate their medical records to the database. The goal over the next few years is to create an unprecedented database of high quality and granularity with tens of thousands of de-identified glioblastoma patients, with open-access to qualified academic researchers. In addition, this powerful 'real world experience' database will be useful for pharma/biotech companies, and may also facilitate FDA drug approvals for glioblastoma.

INNV-10. PERIPHERAL MYELOID CELL pd-l1 IS A BIOMARKER FOR HIGH-GRADE INTRACRANIAL MALIGNANCY

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INTRODUCTION: Immunosuppression is a hallmark feature of primary and metastatic malignancies in the brain. Expansion of suppressive myeloid cells expressing PD-L1 has been demonstrated in circulation of GBM patients. Similar findings have been reported in numerous advanced malignancies, but the presence of suppressive myeloid cells is rarely seen in patients with benign tumors. We hypothesized that expression of PD-L1 on peripheral myeloid cells may be used to differentiate malignant from non-malignant brain tumors prior to tissue diagnosis. METHODS: Peripheral blood was collected from 189 patients undergoing surgical resection of tumors, including low grade gliomas (n=5), high grade gliomas/GBM (n=76), benign meningiomas (n=18), atypical meningiomas (n=25), anaplastic meningiomas (n=10), non-metastatic early stage NSCLC (n=15), and brain-metastatic NSCLC (n=34), as well as from healthy donors (n=6). Immunosuppressive myeloid cells ($CD45^+CD11b^+CD163^{+/-}PD\text{-}L1^+$) and myeloid-derived suppressor cells ($CD11b^+CD33^+HLA\text{-}DR^{lo}PD\text{-}L1^{+/-}$) were quantified through flow cytometry. RESULTS: Peripheral myeloid PD-L1 positivity was significantly elevated in patients with high grade vs. low grade gliomas (19.7% vs. 5.7%; $p<0.0001$), anaplastic vs. other meningiomas (12.6% vs. 6.2%; $p<0.01$), and brain-metastatic vs. non-metastatic NSCLC (11.3% vs. 4.1%; $p<0.0001$). Using a threshold of 10%, peripheral monocyte PD-L1 expression was found to be predictive of high grade malignancy with a positive predictive value of 95.2%. Sensitivity was 57.1% and specificity was 96.2%. MDSC abundance was also significantly increased in patients with high grade tumors (36.3% in gliomas, 14.4% in meningiomas, 18.0% in metastatic NSCLC) compared to lower grade tumors (11.4%) and healthy donors (5.7%). CONCLUSION: Expansion of $PD\text{-}L1^+$ myeloid cells is strongly associated with the presence of intracranial malignancy, with a predictive value in excess of 95% for patients with an intracranial mass. In cases where pre-biopsy knowledge of a high-grade malignancy may change the surgical approach to diagnosis and treatment, blood analysis for myeloid PD-L1 may be considered as a first step.

INNV-11. THE HONOR PROJECT: A NEURO-ONCOLOGY TEAM ADDRESSES GRIEF

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BACKGROUND: Neuro-oncology team members connect with patients through direct patient care, correspondence and the electronic medical record. Hence, relationships develop between patients and various team members. The prognosis for patients diagnosed with a malignant glioma remains grim. Consequently, team members have constant exposure to death. When a patient dies, team members experience loss but often have no formal opportunity to grieve. Rachel Naomi Remen, MD notes "We burn out, not because we don't care, but because we don't grieve... because we have allowed our hearts to become so filled with loss that we have no room left to care." Grief is unavoidable and may affect job satisfaction and productivity if left unaddressed. METHOD: The Honor Project was designed as a meaning-centered team intervention to give voice to grief through ritual. An "altar" is mounted in a staff-only area. When notified of a death, the team member is invited to pause and mindfully write the name on a card and place it on the altar. The team gathers monthly to read the names aloud and share stories, poems, meditations, tears and laughter. Chocolate is gifted to "*ease the bitterness of death and remind us of the sweetness of life.*" The team listens to a closing song as they take rest before returning to work. Team members were surveyed after six months. RESULTS: 100% of respondents reported being better able to cope with sadness related to patients' deaths and feeling less alone in their own grief. The Honor Project provided a meaningful experience at work for 100% of respondents who said it made a difference knowing management allows time during work to give voice to their grief. CONCLUSION: Identifying work-related loss normalizes grief, enhances coping, helps team members honor their own feelings of loss and creates a supportive work community.

INNV-12. A QUALITY IMPROVEMENT PROGRAM FOR GLIOBLASTOMA PATIENT CARE QUALITY IN TWO ACADEMIC TERTIARY U.S. NEURO-ONCOLOGY CENTERS

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BACKGROUND: To address needs for quality improvement (QI) initiatives in glioblastoma (GBM) patient care, we are conducting a QI program in 2 major U.S. neuro-oncology centers. METHODS: At baseline, we retrospectively reviewed EMRs for 100 GBM patients (50 in each center), assessing physicians performance of quality-based and NCCN guideline-directed biomarker testing and diagnostic procedures, time from diagnosis to tests/treatments, tumor board reviews, clinical trial enrollment, GBM treatments, and patient-centered measures. Multidisciplinary GBM teams in each center are participating in QI interventions including feedback on baseline EMR results and developing action plans for improvement. Post-intervention EMRs will be retrospectively reviewed for 50 GBM patients in each center. RESULTS: At baseline, for 100 GBM patients diagnosed between 2012–17, median age was 63 years (26–89), median KPS was 80 (40–90), 22% were female, 82% were Caucasian, 72% were referred from community settings, and 17% were treated at a county hospital with chemotherapy or radiation. Percentages of biomarker tests included: MGMT, 61%; IDH1/2, 80%; 1p19q codeletion, 63%; EGFR, 65%; and TERT, 5%. Mean number of days from diagnosis to tests/treatments were: Postoperative MRI, 2.2; pathology report, 8.6; molecular test report, 8.1; initiation of chemotherapy, 27.7; and radiotherapy, 29.3. Tumor board reviews and clinical trial discussions were performed in 55% and 84% of patients, respectively. Percentages of patient-centered care practices included: interdisciplinary care coordination, 92%; discussion of prognosis, 79%; and timely hospice involvement for patients without treatment options, 40%. Higher incidence of selected biomarker tests performed was significantly associated with male sex, Caucasian race, higher KPS grade, and clinical trial discussion. CONCLUSIONS: The methods and findings from this QI program on GBM are relevant to designing scalable initiatives across community-based and academic neuro-oncology centers. Our presentation, which will include post-intervention results, will address practical applications for improving quality-based and guideline-directed practices for patients with GBM.

INNV-13. ALLELE: A CONSORTIUM FOR PROSPECTIVE GENOMICS AND FUNCTIONAL DIAGNOSTICS TO GUIDE PATIENT CARE AND TRIAL ANALYSIS IN NEWLY-DIAGNOSED GLIOBLASTOMA

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BACKGROUND: Advances in genomic profiling, together with a better understanding of cancer genetic drivers have generated opportunities for precision medicine trials in newly diagnosed glioblastoma (GBM). ALLELE is a consortium to create independent infrastructure for prospective genomics and functional diagnostics to support biomarker-driven trials in GBM. METHODS: Multi-center prospective study of molecular profiling in newly diagnosed GBM. Clinical (CLIA) tumor/normal whole exome sequencing (WES) and genome-wide copy number array (CNA) were performed. Primary objective: to evaluate the feasibility of genotyping tumors with a turnaround time allowing prospective data use in prospective trials. Secondary objectives included developing infrastructure for novel functional biomarker assays and investigating the clinical yield of tumor/normal WES and CNA in GBM. RESULTS: As of 5/1/18, 65 patients with GBM enrolled among 7 sites. Median age was 59. WES and CMA were completed in 60 patients, with a median time between tissue submission and reporting of 22 days (range 15–35). 33 patients were enrolled in INSIGhT, a randomized platform adaptive trial comparing standard of care versus adjuvant CC-115, neratinib or abemaciclib in MGMT unmethylated newly diagnosed GBM (NCT02977780). In each arm, pre-defined biomarkers (EGFR, PI3K and CDK) will be evaluated for their ability to predict outcome. Potentially actionable findings were identified in 30 patients, and included EGFR amplification, mutations of BRAF, FGFR1, IDH1/2 or FGFR3 or MET fusion. Four tumors were reclassified based on genomics. Functional biomarker assays were developed for real-time evaluation of pharmacodynamic responses in ex vivo models. Updated results of exploratory biomarker analyses will be presented at the conference. CONCLUSIONS: Real-time WES, copy number arrays and functional diagnostics are feasible in newly diagnosed glioblastoma, and can support a variety of biomarker-driven trials. Genomic analyses conducted in a prospective manner can inform subsequent clinical trial analysis aiming at matching outcome with tumor genotyping.

INNV-15. ANALYSIS OF CHALLENGES TO ACCRUAL IN CLINICAL TRIALS FOR NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: Glioblastoma (GBM) is the most common glioma, with an incidence of 3.2 per 100,000 adults, and approximately 12,500 new diagnoses each year in the US. With limited treatment options, there is a constant push to improve patient outcome by testing experimental therapies in clinical trials. However, it is estimated that only 8%-11% of patients with newly diagnosed GBM enroll to clinical trials. METHODS: In an effort to better prioritize patients into appropriate clinical trials and understand barriers to trial enrollment, a weekly Tumor Planning Conference was instituted at MD Anderson Cancer Center in August 2016 to discuss eligibility of each patient with newly diagnosed GBM for available clinical trials. RESULTS: We performed a retrospective review of 112 patients presented over the course of 17 months; data collected included age, gender, date, location and extent of surgery, post-operative day at time of conference, available molecular data and the date(s) it was reported, patients residence and Karnofsky Performance Scale score. A total of 2 to 4 clinical trials for newly diagnosed GBM were available for enrollment at any one time during the analysis. Of the total patients consented for trial participation, 8 were ultimately enrolled and treated into clinical trials (7.14%). The primary reason for no trial participation was study-specific eligibility/ineligibility criteria (such as lack of necessary molecular markers) (46.3%), followed by being out-of-window for trial enrollment (27.4 %). CONCLUSIONS: The weekly tumor planning conference did not improve the number of accruals to trials for newly diagnosed GBM compared to what has been previously reported. However, it provided valuable insights about barriers to trial enrollment, such as the importance of timely outside referrals, efficient new patient scheduling, and need for expedited trial-specific molecular testing.

INNV-16. miRNA SIGNATURE DERIVED FROM GBM PLASMA EXOSOMES AS A DIAGNOSTIC BIOMARKER

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Gliomas including glioblastoma (GBM) are the most common malignant brain tumors. Glioma extracellular vesicles (EVs), especially plasma exosomes, have biological effects such as mediating immunosuppression and contain signature tumor-specific cargo that could serve as liquid biopsies. Increasing interest in molecular biomarkers to determine patient prognosis in GBM has suggested that EV miRNA-based signatures may be able to predict progression-free and overall survival, differentiate normal donors from GBM patients, and distinguish true progression from treatment-related pseudo-progression. We have established a simple two-step technique, using density gradient ultracentrifugation (DGU), to isolate plasma exosomes from glioma patients and normal donors. One-step DGU (90 minutes/24,000 RPM) efficiently isolated exosomes from plasma, while a second DGU (16 hours/24,000 RPM) efficiently concentrated exosomes for subsequent cargo analysis, including miRNA signature analysis. Purification of total RNA, including miRNA, was performed on plasma exosomes from normal donors (n=8) and GBM patients (n=8) using the miRNeasy kit (Qiagen). Next generation short non-coding RNA sequencing samples was performed by Illumina HiSeq 2000 and revealed many differentially expressed miRNAs in GBM patients with high fold change/low false discovery rates compared to normal donor plasma exosomes. In order to test the diagnostic accuracy of the proposed technique for the differentiation of GBM patients and normal donors, ROC analysis was performed based on the top 30 differentially expressed miRNA samples. The area under the ROC curve (AUC; a figure of merit to determine the optimal miRNA signature) was 0.968. In addition, multiple novel miRNAs and other short non-coding RNA species (Y-RNA, piRNA, snoRNA) were found with some differential expression. In conclusion, miRNA sequencing from plasma exosomes shows marked differential miRNA expression between healthy donors and GBM patients. These findings as well as additional differentially expressed short non-coding RNA species suggest plasma EVs may serve as a robust platform to develop GBM liquid biopsies.

INNV-17. TUMOUR TREATING FIELDS: ACCEPTABLE TO A UK POPULATION?

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BACKGROUND: Tumour treating fields (TTFields) are an innovative anticancer treatment that uses alternating electrical fields to disrupt cell division. In a randomized controlled trial, TTFields significantly improved newly diagnosed Glioblastoma patient survival. The device may be thought to be taxing for patients, with almost constant electrode application and battery use. Methods were explored to reduce cost and examine acceptance. METHODS: Three United Kingdom (UK) centres were offered support for trialing the treatment. Patients with proven MGMT unmethylated glioblastoma, had completed radical chemoradiotherapy, had a performance score > 70, and had social support (for placing the electrodes), where approached. Monthly assessments of compliance and quality of life (QOL) (EORTC BN 20), and quarterly MRIs and tolerability questionnaires were completed. RESULTS: Oncologists in one centre declined to support any use of the device. 8 patients were approached in the other two centres, 5 accepted, and 1 further patient had treatment started elsewhere. A UK politician developed glioblastoma, used the device, and this was followed by positive press reports. Clinicians were trained in the use and interface with the equipment. All patients tolerated the treatment and used for 85% or more. The reason for patient refusal was monthly travelling for compliance checks. Adverse comments related to the mobile battery weight. Two patients had skin irritation, and two found that showering was affected. Four patients have progressed, but maintained stable QOL scores. Two patients have died. CONCLUSIONS: TTFields is well tolerated but requires

patient dedication to use the device for at least 18 hours per day and in our pilot access study was acceptable to a UK population. Recent positive press support aided recruitment. A significant reduction in cost is required to allow routine UK use.

INNV-18. CAREGIVER SUPPORT IN THE NEURO-ONCOLOGY CLINIC; IDEAS TO ADDRESS THE NEEDS OF CAREGIVERS AS PART OF THE PATIENT CAREPLAN

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BACKGROUND: Support of the neuro-oncology caregiver is a critical element of patient care, and enhances the well-being of the caregiver and patient. At UCSF, the Gordon Murray Caregiver Program has a RN, social worker, and coordinator team dedicated to providing tailored services to caregivers across the disease trajectory. For programs where resources may be more limited, it is a challenge to address caregiver needs as well as the clinical care of the patient. We identified the areas of highest need and program utilization as a focus point for those looking to expand into caregiver support. METHOD: We reviewed the experience in our program in providing services to over 800 caregivers over the last 5 years. We noted domains of highest need, time points along the illness trajectory where caregivers utilized the most services and noted the specifics of the care delivered. RESULTS: High need areas for caregivers include the need for emotional support, health information and health navigation. Significant time points noted for most referrals for care were at diagnosis, disease progression, and/or transition to hospice. Emotional needs were addressed through consultations with Caregiver Program members and/or a caregiver specific support group. Health information was provided using disease specific and illness trajectory specific materials. Navigation was facilitated with nurse or social worker consultations. CONCLUSION: Addressing the needs of the caregiver starts by acknowledging the caregiver as part of the care team from the time of diagnosis. Neuro-oncology programs can leverage existing resources to provide caregivers support, such as health information appropriate to the clinical status of the patient, connections to caregiver specific community resources, support in developing positive coping strategies and host a caregiver specific support group. These are efficient and low cost methods to work on addressing caregiver needs.

INNV-19. PLASMA EXTRACELLULAR VESICLE GLIAL FIBRILLARY ACIDIC PROTEIN AND TAU AS BIOMARKERS FOR BRAIN CANCER

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Early detection of brain cancer remains a challenging proposition. We applied a novel alternating current electrokinetic (ACE) chip device that relies on the dielectrophoretic (DEP) force to isolate and analyze the proteomic content of extracellular vesicles (EV) derived from undiluted patient plasma to identify biomarkers for early detection of brain tumors. EVs derived from cultured cells or plasma samples were isolated using the ACE chip. The concentration of intra-vesicular glial fibrillary acidic protein (GFAP) and Tau was determined using immunofluorescence staining. Improvement in model prognostication was quantified using net reclassification improvement (NRI>0) and integrated discrimination improvement (IDI). EVs secreted by cultured brain tumor cells (brain metastasis, meningioma, and glioma) harbored high levels of GFAP and Tau. We isolated EVs from plasma collected from brain tumor patients (5 meningiomas, 5 metastases, 6 gliomas) and 17 non-cancer controls. Compared to controls, plasma EVs from brain tumor patients exhibited greater fluorescence for GFAP (1.94 ± 0.139 vs. 1.28 ± 0.042, p<0.0001) and Tau (4.92 ± 0.43 vs. 1.79 ± 0.11, p<0.0001). Immunofluorescence did not differ between tumor types. Elevated EV GFAP was associated with a sensitivity of 88%, a specificity of 92%, and a AUC of 0.931 (95% CI 0.84–1.022) for brain tumor detection. Similarly, elevated EV Tau was associated with sensitivity of 94%, specificity of 94%, and AUC of 0.948 (95% CI 0.846–1.05) for brain tumor detection. The combination of EV GFAP and Tau improved test discrimination relative to GFAP alone (NRI>0 1.66, 95% CI 1.19–2.13, p<0.001; IDI 0.26, 95% CI 0.11–0.41, p<0.001) or Tau alone (NRI>0 1.54, 95% CI 1.03–2.05, p<0.001; IDI 0.18 0.038-0.32, p=0.013). We have provided proof-of-principle studies to demonstrated the utility of a novel DEP-based technology for minimally invasive brain cancer detection using undiluted patient plasma.

INNV-20. UTILITY OF TELEHEALTH FOR SPECIALTY NEUROFIBROMATOSIS (NF) CARE

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BACKGROUND: Telehealth allows for evaluation and management to be delivered over long distances, which may particularly benefit patients with rare neurological diseases. METHODS: We performed a retrospective cohort study of patients receiving telehealth-based care in our NF clinic, and used ArcGIS to calculate driving distance and time from patients home to clinic. RESULTS: 109 patients (70 female, median age: 37 years) had telehealth visits from May 2016-March 2018, eighteen (17%) of whom had multiple telehealth visits. Patient diagnosis was 33% NF1, 42% NF2, 8% Schwannomatosis, and 17% other, compared to our clinic population of 58% NF1, 31% NF2, 6% Schwannomatosis and 5% other. Only 7 patients (6%) were pediatric, compared to 15% pediatric population in our NF clinic. Telehealth visit indication was 62% routine follow up, 26% new test result follow up, 6% evaluation of a new problem, and 6% on-therapy follow up. The plan developed by telehealth visits included 24% new radiology ordered, 7% new medication ordered, 18% new specialty consultation ordered, and 51% no change in previous plan. Telehealth saved patients a median round trip drive of 108 miles (IQR 388 miles) and a median driving time of 170 minutes (IQR 292 minutes). CONCLUSIONS: Telehealth improved NF specialty care through more accessible routine follow up, and urgent evaluation of new symptoms in 6% of patients. Telehealth visits led to new testing, referrals, or medications in 49% of cases, showing that telehealth fulfills a distinct need for services while saving patients time and travel. Adult patients were overrepresented among telehealth cases compared to our general clinic volume, suggesting that in-person examination of children may be preferred by NF providers or families. Given difficulties in access to specialty care, telehealth offers an opportunity to extend care for patients with rare neurological diseases who live at a distance from specialty centers.

INNV-21. AN OVERVIEW OF NIGERIAN NEURO-ONCOLOGY SCHOLARLY OUTPUT

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BACKGROUND: Nigeria is the most populous country in Africa and home to the continent's largest economy. In this lower middle income county there is a shortage of optimal neurological/neurosurgical oncology care for a population of 184 million. METHODS: A systematic review of the literature was performed using Google Scholar, PubMed, and African Journals Online, searching for articles related to neuro-oncology in Nigeria. The following search terms were used: Neuro-oncology, neurooncology, brain tumor, glioma, meningioma, central nervous system tumor, management, practice and Nigeria. Manuscripts were reviewed for relevance and categorized. RESULTS: 63 relevant articles were identified comprising original research in basic science (N= 1), clinical science (N = 59), and reviews (n=3).Retrospective case series were the most common types of publications from amongst these categories. Categorizing according to histology articles focused on meningioma (N=12), pituitary tumors (N=10), glioma (N=7), CNS metastases (N=6), multiple histologic types (N=25) and other types of tumors (N=3). Only 8 pediatric neuro-oncology publications were noted amongst these. Two manuscripts specifically addressed issues on neuro-oncology clinical practice in Nigeria. Twenty-six were published in Nigeria journals, 9 in US journals, and twenty-eight published in other countries. An increase in number of publications over time peaking in 2015 (14 manuscripts) was noted. The most frequent affiliation of authors was University of Ibadan (26), University of Nigeria Teaching Hospital, Enugu (8), and Memfys Hospital for Neurosurgery (6). Two manuscripts were co-authored with American-based authors. CONCLUSION: There is a small but growing scholarly literature in neuro-oncology from Nigeria. However, there continues to be room for growth in neuro-oncology research output. With its large population of patients much can be learned. While there are logistical impediments to both patient care and research in neuro-oncology in Nigeria, there is promise for favorable advancements.

INNV-22. LIQUID BIOPSY DETECTION OF GENOMIC ALTERATIONS IN PEDIATRIC BRAIN TUMORS FROM CELL FREE DNA IN PERIPHERAL BLOOD, CSF, AND URINE

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Brain tumors often require invasive neurosurgical procedures for diagnosis and tissue acquisition to guide precision medicine. Detection of tumor-derived cell-free DNA (cfDNA) would facilitate tumor profiling without risk of surgery-related morbidity and provide a means of tumor surveillance. Our aim is to validate a sample collection procedure and develop highly sensitive and specific methodologies to detect tumor-derived cfDNA in CSF, plasma and urine from pediatric patients with brain tumors to identify clinically relevant genomic alterations. Since July 2016, we have prospectively collected blood, urine and CSF samples from 235 patients across all histological subtypes at Dana-Farber Cancer Institute/Boston Childrens Hospital. In parallel, we sequenced the tumors to identify tumor-specific genomic alterations. We optimized a method to process cfDNA and perform ultra-low pass whole genome sequencing (ULP-WGS) using unique molecular identifiers, confirming we can reliably construct sequencing libraries from CSF-, plasma- and urine-derived cfDNA. ULP-WGS has also been used to assess sequencing library quality, copy number variations (CNVs) and tumor fraction. The vast majority of samples undergoing ULP-WGS exhibited no CNVs, consistent with either absence in the tumor or low levels of tumor-derived cfDNA. To distinguish between these, we developed a hybrid capture sequencing panel covering 46 genes, allowing identification of specific mutations and fusions more common in pediatric brain tumors. We are currently assessing the specificity and sensitivity of our assays for detection of tumor-derived cfDNA by sequencing samples with matched tumor NGS available. Furthermore, we are identifying cases with multiple time points of cfDNA, for which data from paired pre- and post-treatment tumors are available, to apply. Our results will provide insights regarding the feasibility of cfDNA assays to guide clinical care in children with brain tumors.

INNV-23. SAFETY AND ADVERSE EVENT PROFILE OF TUMOR TREATING FIELDS IN ELDERLY PATIENTS A POST-MARKET SURVEILLANCE ANALYSIS

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INTRODUCTION: Tumor Treating Fields (TTFields) are an established modality for glioblastoma (GBM) treatment administered through the portable Optune system. The efficacy of Optune in newly diagnosed GBM was previously demonstrated in the EF-14 phase 3 trial (n=695). Optune plus temozolomide significantly improved survival in all patient subgroups compared to temozolomide alone, which included those patients 65 years of age (median OS: 17.1 vs 13.7 months). Here, we report post-marketing surveillance data from elderly patients treated with Optune. METHODS: A review of adverse events in patients 65 years of age. Post-market surveillance data were analyzed based on the MedDRA body system (system organ class) preferred terms. RESULTS: A total of 8025 patients were treated with Optune (newly diagnosed glioblastoma 46.1%, recurrent glioblastoma 46.2%, other 7.7%). Of those, 2574 patients (32.1%) were 65 years of age at the time of Optune treatment. 46% of these elderly patients reported 1 adverse event. Skin reaction was the most prevalent adverse event and occurred in 28% of patients. Similarly, 47% of patients 18 and <65 years of age (N=5421) experienced at least 1 adverse event and 27% developed skin reaction. The remaining adverse events profile was fairly equally distributed between both age groups. CONCLUSION: This retrospective analysis demonstrates that Optune is used in patients 65 years of age or older. The adverse event profile in this group of elderly patients is comparable to patients younger than 65 years. The most common adverse event registered was skin reaction, which was also found in the phase 3 EF-11 trial for recurrent GBM and in the EF-14 trial for newly diagnosed GBM patients. In summary, these results underline the safety profile of Optune.

INNV-24. SAFETY OF TUMOR TREATING FIELDS IN GLIOBLASTOMA PATIENTS WITH IMPLANTED NON-PROGRAMMABLE AND PROGRAMMABLE SHUNTS, AND PACEMAKERS/DEFIBRILLATORS: 6.5-YEAR UPDATED RETROSPECTIVE ANALYSIS

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BACKGROUND: Tumor Treating Fields (TTFields) are a noninvasive, loco-regional, anti-mitotic cancer treatment approved in the U.S. for adults with recurrent and newly diagnosed glioblastoma (GBM). TTFields (low intensity, intermediate frequency, alternating electric fields) are delivered by a portable medical device (Optune®). GBM patients may develop hydrocephalus and benefit from subsequent shunt placement. Safety information on concomitant use of TTFields with implanted devices in GBM patients will identify potential interference with the functioning of these devices. METHODS: A review of the clinical information for GBM patients treated with TTFields in the U.S. between November 2011 and May 2018 identified 79 patients with non-programmable shunts (NPS), 11 with programmable shunts (PS), and 14 with pacemakers/defibrillators (PM/DF). The safety data obtained from post-market surveillance in all 104 patients were analyzed to identify implantable device-related adverse events. RESULTS: Adverse events (AEs) reported for these 104 patients did not reveal any new safety concerns on concurrent use of Optune with implanted devices. Infections or infestations (bronchitis, diverticulitis, infection NOS, meningitis, sepsis, shunt infection, upper respiratory and urinary tract infections, wound infection) were reported in 13/79 (15.2%) of NPS patients and in 4/11 (36.4%) of PS patients. No arrhythmia or other cardiac adverse events were reported in the 14 patients with PM/DF. Skin irritations were seen in 4/79 (5.1%) of NPS patients. Neurological symptoms (35.4%), which included convulsions (15.2%) in the NPS patients were related to the underlying tumor rather than TTFields treatment. Hydrocephalus was seen in 6/79 (7.6%) NPS and 2/11(18.2%) PS patients but were not related to shunt malfunction. CONCLUSIONS: No unexpected safety issues were observed in the 104 patients analyzed in this updated report. Further evaluation of the concurrent use of TTFields with programmable shunts and pacemaker/defibrillators should include bench testing of the compatibility of these devices to TTFields.

INNV-25. IMPROVING QUALITY OF CARE OF MENINGIOMA PATIENTS: INITIAL EVALUATION OF ISSUES IN CARE TRAJECTORIES ACCORDING TO THE PLAN-DO-STUDY-ACT CYCLE

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BACKGROUND: Meningioma are common benign intracranial tumours, that can cause severe neurological and daily functional problems, sometimes requiring lifelong care and support. Current practice and guidelines lack focus on these long-term sequelae. Value-Based Healthcare (VBHC) initiatives aim to increase the value of care for patients, but continuous evaluation and improvement of these initiatives is required. According to the Plan-Do-Study-Act (PDSA) cycle, a model for continuous healthcare evaluation and improvement, we explored issues in meningioma care trajectories, possible high impact initiatives to improve these issues, and the implementability of these initiatives. METHODS: Previously, issues in the meningioma care trajectories were identified through a survey by The Netherlands Comprehensive Cancer Organisation. Using a grounded theory approach, a thematic framework was constructed based on this data and used for further data collection through three semi-structured interviews with patient-partner dyads (i.e. pairs) and four focus groups with patient-partner dyads and healthcare providers (both two focus groups). RESULTS: Issues in meningioma care trajectories reported by patient-partner dyads and healthcare providers were related to information, care and support, logistics, and diagnostics and treatment. Most important issues were lack of information about intervention and outcomes, and lack of support after treatment. A reported solution for many issues was the incorporation of a case manager. Other possible solutions were implementation of routine use of patient-reported outcome measures (PROMs) or a rehabilitation program, and formalization of these care trajectories. The most important barriers for these solutions were budget, capacity, ICT infrastructure, qualified personnel, and know-how. CONCLUSION: Implementation of a few simple solutions (e.g. case manager) may improve the majority of experienced issues in meningioma care trajectories. Information on the identified barriers and facilitators can be used to ensure successful implementation of these practice changing initiatives, which need iterative evaluation in PDSA cycles.

INNV-28. EXTENT OF RESECTION IN NEWLY DIAGNOSED GLIOBLASTOMA: INCORPORATING CLINICAL AND MOLECULAR DATA TO PREDICT OUTCOME

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BACKGROUND: The benefits of increasing extent of resection (EOR) for both overall survival and progression-free survival (PFS) in glioblastoma has been well documented. However, models predicting surgical outcomes have failed to incorporate a patients IDH status, a known prognostic factor. We isolate the impact of IDH on surgical outcomes. We determine the effect modification of increasing EOR and decreasing residual tumor volume (RTV) on IDH status. METHODS: We performed a retrospective cohort study of 98 patients with glioblastoma who had undergone either biopsy or surgical resection. Tumor volumes were determined by volumetric analysis. Univariable and multivariable Cox PH Regression models were built using overall survival and PFS as endpoints. RESULTS: Increasing EOR and decreasing RTV were both associated with prolonged overall survival and PFS. When IDH status was added to multivariable models, the model utilizing RTV provided a slightly better fit compared to EOR. An interaction term between RTV and IDH status was characterized, such that at low RTVs the prognosis of an IDH mutant is significantly better than that of an IDH wild-type, an effect that is less important as RTV increases. The significance of this term was confirmed by improved fit upon insertion into multivariable models. CONCLUSION: Minimizing RTV and increasing EOR are important prognostic factors for both IDH wild-type and IDH mutant glioblastoma. The protective benefit of the IDH mutation at lower RTVs suggests these patients are the best candidates for aggressive surgical resection.

INNV-29. EXPERIENCE WITH TTFIELDS (OPTUNE®) IN PEDIATRIC HIGH GRADE GLIOMA PATIENTS IN ISRAEL

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INTRODUCTION: TTFields are an established treatment for glioblastoma (GBM) in adults and have been shown to prolong OS, PFS and long-term survival with minimal side-effects. TTFields are not yet approved for children and the device (Optune®) was provided on a compassionate basis to all patients in this case series. We report our experience with TTFields at 4 major pediatric oncology centers in Israel. METHODS: Since September 2017 Optune has been offered to 7 pediatric patients of whom 5 (3 female, 2 male) gave consent immediately. These patients were aged 11.1 - 17.7 years at time of diagnosis. 4/5 had a midline diffuse glioma H3.3K27M positive and started Optune together with temozolomide following biopsy/subtotal resection and radiation. The fifth patient had a gross total resection of a right parietal GBM positive for the H3.3G34R mutation. He was treated with radiation and adjuvant temozolomide and had a multifocal right sided relapse at 20 months, and was then treated with Avastin and Optune. RESULTS: Two patients reported device-related mild itchy skin, which was easily treated topically. Four patients continued all normal activities despite carrying the device during school attendance, trips abroad and even at a waitressing job. One male refused to attend school and continues home learning. Three patients had programmable shunts, without any interference with Optune. Compliance was >90% in 3 patients, 80% in 1 individual and 60% in the relapsed patient. 2/5 patients have stopped treatment due to progression after 86 and 142 days on Optune, however all patients are still alive at the time of the report. CONCLUSION: In summary TTFields is a feasible and tolerable treatment even in children as young as 11 years. TTFields had no additional systemic side-effects and showed good acceptance rate. Further studies in pediatric patients are needed to evaluate efficacy in pediatric high-grade glioma.

INNV-30. TUMOR TREATING FIELDS AND RADIOTHERAPY FOR NEWLY DIAGNOSED GLIOBLASTOMA: SAFETY AND EFFICACY RESULTS FROM A PILOT STUDY

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BACKGROUND: Tumor Treating Fields (TTFields) are a non-invasive, loco-regional, anti-mitotic treatment comprising low intensity alternating electric fields. In the phase 3 EF14 study in newly diagnosed glioblastoma (ndGBM), TTFields/temozolomide significantly improved survival versus temozolomide alone. TTFields increased glioma cells undergoing cellular death following radiotherapy (RT), suggesting a radiosensitizing effect that enhances RT efficacy. This single center study is the first to investigate TTFields concomitant to RT in ndGBM. METHODS: Enrolled ndGBM patients (N=10), KPS 70 had recovered from maximal debulking surgery or biopsy. Patients started TTFields prior to or at the time of RT, and were on stable/ decreasing corticosteroids doses for 7 days pre-enrollment. Patients received TTFields (200 kHz; 18 hours/day) with daily removal of transducer arrays during RT, temozolomide (75 mg/m2/daily for 6 weeks) and 60 Gy RT. Endpoints included safety of TTFields/RT (primary) and preliminary efficacy. RESULTS: Patients were 59 years (42–71), KPS 90 (80–100) and mostly male (80%). Five patients (50%) underwent gross total resection while rest had biopsy only. Median dose of RT was 60 Gy (range 52–60 Gy). Six patients (60%) reported have an adverse event (AE) to-date. The most common AE was TTFields-related skin toxicity in 4 (40%) patients; none were severe. All other AEs reported occurred in only one patient and be attributed to underlying disease or chemotherapy. Two reported serious AEs (seizures and general deterioration) were considered unrelated to TTFields. Median PFS was 10.5 months (95%CI 2.67–10.5). PFS rate at 6 months was 80% (95%CI 40.9–91.6). CONCLUSION: The proportion of TTFields-related skin toxicity (40%) was similar to that reported in phase III study (52%), where patients started TTFields > 4 weeks after RT. No other TTFields-related toxicities were reported, nor was there an increase in RT- or temozolomide-related toxicities from combining TTFields with these therapies. Preliminary survival data are encouraging.

INNV-31. USER EXPERIENCE WITH NEW, AESTHETICALLY IMPROVED TRANSDUCER ARRAYS FOR DELIVERY OF TUMOR TREATING FIELDS FOR GLIOBLASTOMA

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INTRODUCTION: Tumor Treating Fields (TTFields) are low-intensity, intermediate frequency alternating electric fields for glioblastoma. TTFields are administered using the Optune® system, comprised of two main components an electric field generator and insulated transducer arrays (TAs). The current TAs consist of 2 opposing pairs of TAs having ceramic disks arranged within a white adhesive bandage, designed to hold the TAs tightly to the shaved scalp. In response to patient and healthcare provider requests, NovocureTM evaluated tan colored TAs to minimize the stark appearance of white TAs for patients, making the TAs more amenable for use in public settings. MATERIALS AND METHODS: This study was carried out in 25 newly diagnosed and recurrent GBM patients in Germany receiving Optune. Patients with KPS >70, caregiver support and skin intact at study initiation were asked to complete a questionnaire, which assessed patient satisfaction with the comfort, aesthetics and convenience associated with the tan TAs. Patients reported responses to questionnaire at day 0 (white arrays), days 7 and 28 (wearing the tan arrays). Median patient reported changes from baseline to Day 28 in responses in the questionnaire were compared using a Wilcoxon signed rank test. RESULTS: Patients reported the color of TAs to be an important attribute ($p<0.011$). The tan arrays were less conspicuous than white arrays ($p<0.0052$). Participants in Germany found tan color was neither too dark nor too light ($p<0.001$). Participants felt that wearing the tan arrays were significantly cooler than the white arrays. CONCLUSION: Optune is designed to be integrated into patients daily life. Results from phase 3 clinical trials showed a correlation between efficacy of TTFields and daily treatment duration. Therefore, improvements addressing patients user experience with the tan colored TAs, which minimize calling attention to the patients medical condition, may help to further improve treatment compliance and patient satisfaction.

INNV-32. DETERMINATION OF INTERLEUKIN 10 IN THE DIAGNOSIS OF PCNSL

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BACKGROUND: Primary central nervous system lymphoma (PCNSL) is an extranodal lymphoma and accounts for 5% of intracranial neoplasms. The diagnosis is made by biopsy, but false negative rates of 11–30% have been reported usually due to patients being previously treated with steroids or insufficient sample obtained. In addition the population in which it occurs are patients older than 50 years, which present comorbidities and sometimes the surgical procedure represents a high risk. METHODS: Patients with a confirmatory histopathological study and who also had a lumbar puncture as part of their approach were included. IL-10 was determined by immunoassay technique (ELISA) and it was taken as normal range 2–4 pg/dl. RESULTS: 39 patients (16 women and 23 men) with confirmed diagnosis were included, with an average age of 50.1 years. The mean CSF proteins was 76.5 mg/dl, hypoglycorrhachia was present in 20.5%, the mean CSF cells were 15.9, mean IL-10 was 120 pg/dl with 94.8% higher than 4 pg/dl. Correlation coefficient between IL10 level and overall survival was performed without finding any significant results. CONCLUSIONS: The determination of IL-10 is a less invasive determination that can allow the

diagnosis of PCNSL, it is an alternative where the diagnosis is not clear, the biopsy is risky or the result is not conclusive; however, it is necessary to determine its value in other pathologies to evaluate its sensitivity and specificity.

INNV-33. IMPLEMENTATION OF ELECTRONIC DATA CAPTURE FOR USE IN NATURAL HISTORY STUDIES: UTILITY OF CENTER FOR CANCER RESEARCHS (CCR) LABMATRIX AND SCRIBE SYSTEMS FOR THE NEURO-ONCOLOGY BRANCH NATURAL HISTORY STUDY (NOB-NHS)

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Accurate and prompt data collection is vital to clinical research. Traditionally, patient reported outcomes (PROs) have been captured through paper/pencil format and clinical data by retrospective clinical document review. Limitations include issues of errors, data omissions and monetary cost. We report an innovative tool for data collection. The primary objective of the NOB-NHS is to provide longitudinal collection of data including clinical status and PROs. Data was initially recorded by data analysts from review of the medical record in nine forms housed in CCRs Labmatrix system. All data fields were reviewed by the clinical team for relevance to the research question, redundancy, and timing of data collection. Free text fields were replaced by pre-determined, standardized pick lists of possible responses. Data fields were then converted into 2 electronic data collection forms (new patient and follow-up) in Scribe, a CCR-developed system for PROs/electronic form data. Feasibility and concordance testing was completed. Lastly, the clinical team was trained to enter data using tablets/laptops in the clinical setting. To date, 400 patients have been registered onto the NOB-NHS, with 222 collected through electronic systems. Links are sent via email to clinicians (nurse practitioners/clinical fellows) and patients that open a survey-style data collection form with drop-down menus and pre-formatted fields. Upon completion, the form automatically syncs to Labmatrix for data deposition and storage. On average, the Study Entry Form is completed in 5 minutes; the Clinic Visit Form in 2 minutes. PROs completion rate is > 95%. Data collected in a systematic and timely manner permits researchers to build stronger queries, reduces time spent on data cleaning, and facilitates statistical analysis. Standardization of the data fields and training of both clinical staff and data managers allows research to be reliable, especially for longitudinal studies such as natural history studies.

INNV-34. INNOVATIVE, INDEPENDENT, PATIENT ADVOCATE ROLE ESSENTIAL THROUGHOUT DRUG DEVELOPMENT IN THE AGE OF SOCIAL MEDIA

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BACKGROUND: A mid-sized biotech company recruiting for high-grade glioma (HGG) clinical trials used the internet for patient awareness. Many patients and families began contacting the company inquiring about trials. METHOD: To offer ethical, informative responses, the company engaged a dedicated, independent patient advocate consultant to interact with the patients and families. This role provides critical separation from the drug company, maintaining patient privacy and preventing recruitment bias. The patient advocate role has evolved into a position that influences all the patient-oriented aspects of the research process. The patient advocate reviews all patient facing documents and provides patient perspectives. The patient advocate informs patients and families about the trial and provides support resources when appropriate. This role requires experience with the brain tumor population and evidence-based knowledge of patient and family needs. The patient advocate communicates with patients and caregivers and helps them navigate through the clinical trials research process. Also, the patient advocate liaises between the drug company and brain tumor advocacy groups. RESULTS: Activities performed since 2014 by Tocagens patient advocate included: A) communicated with > 1200 patients and caregivers through email and phone inquiries; B) reviewed clinical trial protocols, consents and brochures for patient and caregiver suitability; C) participated in the clinical trial patient recruitment team; D) wrote blogs related to living with a brain tumor and understanding clinical trials placed on the company site; E) communicated with brain tumor advocacy groups. CONCLUSION: A patient advocate consultant embedded in a biotech firm provides essential patient-centered perspective throughout drug development. This innovative, independent position provides an interface for patients and families to receive quality, individualized responses to questions. Clinical trial protocols become patient-oriented at times leading to more efficient patient recruitment and trial design. People inquiring about clinical trials receive accurate information, resources with a humane touch.

INNV-35. ROUTINE SEQUENCING OF BRAIN TUMORS UNCOVERS HIGH RATES OF ACTIONABLE OPPORTUNITIES: THE UNC EXPERIENCE

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Since November 2017, the University of North Carolina (UNC) Brain Tumor Program has performed next generation sequencing (NGS) on newly diagnosed brain tumors through the Strata Trial, which uses the StrataNGS assay to evaluate genomic variants in 94 genes. Brain tumors were analyzed from 77 patients revealing 137 total molecular alterations. We defined actionable alterations as: (1) revealing potential enrollment into biomarker-driven clinical trials (regardless of histology); (2) allowing for off-label use of targeted therapy; (3) identifying occult and non-canonical alterations that lead to changes in prognosis and management. At UNC we've identified 26 brain tumors with actionable alterations (34%), and 18 brain tumors (23%) eligible for a nationally-available biomarker-driven trial. These results have provided useful data to inform feasibility of biomarker-driven clinical trials using targeted therapies to treat brain tumor patients seen at UNC. Furthermore, NGS of brain tumors has allowed us to utilize precision medicine in a population of patients with very few treatment options. In particular, we've exploited a BRAF V600E mutation to treat progressive glioblastoma (GBM) with BRAF-targeting agents. The patient rapidly responded to therapy and recovered functionality previously lost while on standard of care therapy. Additionally, NGS identified 1 GBM and 2 anaplastic astrocytoma (AA) patients with MET alterations, which are uncommon in high grade gliomas. Two of these patients enrolled in a biomarker-driven clinical trial at UNC, which would not have been possible without NGS. The patient with GBM has maintained disease control for over 6 months. Our experiences highlight the importance of routine NGS in the management of patients with brain tumors.

INNV-36. A METRONOMIC ANTIANGIOGENIC COMBINATION THERAPY MAY PROLONG SURVIVAL FOR PATIENTS WITH RECURRENT MEDULLOBLASTOMA AND ATYPICAL TERATOID RHABDOID TUMOR

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BACKGROUND: Prognosis of patients with recurrent medulloblastoma and atypical teratoid rhabdoid tumor (ATRT) is dismal despite intensive therapy including high-dose chemotherapy with stem cell rescue. An evolving alternative approach to conventional chemotherapy is to target neovascularisation by interfering with tumor angiogenesis at various levels. We report on 32 patients with recurrent medulloblastoma and ATRT treated with an antiangiogenic combination therapy. PATIENTS AND METHODS: From 11/2006 to 02/2018, 32 patients were diagnosed with recurrent embryonal tumors, 20 with a recurrent medulloblastoma (14 first, 6 multiple recurrences) and 12 with recurrent ATRT (6 first, 6 multiple), three had germ line mutations. Treatment consisted of an antiangiogenic multidrug-regime including IV bevacizumab, oral thalidomide, celecoxib, fenofibrate, and etoposide alternating with cyclophosphamide, and augmented with intraventricular therapy (etoposide and aqueous or liposomal cytarabine). Median age at start of antiangiogenic therapy was 10 (1–24) years for medulloblastoma and 3 (1–12) years for ATRT. RESULTS: As of 05/2018, 10/20 patients with medulloblastoma are alive, eight in CR, six off therapy for 96, 79, 77, 34, 12, and 4 months. 5-year-OS is 54.5 ± 11.2% and 5-year-EFS is 25.0 ± 9.7%. One patient died of an accident in CR 23 months after initiation of antiangiogenic therapy. 6/12 patients with ATRT are alive and in CR for 123, 90, 45, 9, 6 and 3 months after start of antiangiogenic therapy, the first three off therapy. OS for the whole cohort was 47.7 ± 16.6% at 3 years and 31.8 ± 17.1% at 5-years with a median OS of 22.8 months (KI 0.0–66.8). Therapy was generally well tolerated and toxicities were manageable. CONCLUSION: The proposed antiangiogenic regimen is currently being evaluated for medulloblastomas in an international phase II protocol (MEMMAT; ClinicalTrials.gov Identifier: NCT01356290). The same approach seems to be also efficacious in recurrent ATRTs and warrants further evaluation.

INNV-38. THE NEURO-ONCOLOGY BRANCH TRANSLATIONAL RESEARCH IMMERSION PROGRAM: RESULTS FROM TWO YEARS OF DEVELOPMENT AND PARTICIPANT FEEDBACK

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BACKGROUND: Integrated, multidisciplinary research collaborations have the potential to transform cancer research by bridging the gap between highly specialized basic research and clinical strategies to target human disease. Training programs that provide opportunities for exposure to this approach will help promote the translation of basic science discoveries into clinical applications. The Neuro-Oncology Branch Translational Research Immersion Program (NOB-TRIP) was designed to immerse students from diverse scientific and cultural backgrounds into an integrated clinically-centered neuro-oncology research platform. METHODS: Eligible trainees from three separate summer intern programs at the NIH and self-selected for a clinical or basic focus were studied. A trans-disciplinary team developed the NOB-TRIP as a 10-week program consisting of five structured areas of educational focus: didactic lectures; weekly seminars with the NOB Chief on research rigor and career planning; cross-focus observational experiences; integration of wellness; and mentoring in training and public speaking. A post NOB-TRIP survey measured the program's impact. RESULTS/CONCLUSIONS: Twenty-nine interns (18 female; 11 male) participated in the NOB-TRIP. Education levels included high school (3), undergraduate college (17), post-baccalaureate (2), graduate college (3), medical college (3), and post-doctoral (1). Student evaluations were overwhelmingly positive, with two interns returning for a second internship. Students reported acquiring a greater appreciation for the collaborative science and teamwork in a clinically-focused research branch. Patient-caregiver interactions, professional relationships, and the skills required to navigate a difficult conversation were highlighted as invaluable real-world learning experiences. The NOB-TRIP exemplifies the benefits of cross-disciplinary training to spark enthusiasm, confidence, and collaboration among students with diverse scientific interests. These interpersonal and professional skills can drive a desire for scientific discovery and fuel interest in the challenging field of neuro-oncology.

INNV-41. MY STORI -- A SYMPTOM TRACKING AND REPORTING INSTRUMENT MOBILE APPLICATION FOR CENTRAL NERVOUS SYSTEM CANCER PATIENTS

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INTRODUCTION: Managing symptom burden and its impact on the quality of life is an integral part of central nervous system (CNS) cancer patient care. However, keeping track of symptoms and their management is typically done through ad hoc means. Furthermore, reporting is often limited to completing survey instruments as part of the patient follow-up. While general-purpose mobile apps that track medical symptoms exist, they are not geared toward managing and recording the experience of patients with cancer. Cancer-specific apps are emerging but may not cover the entire range of symptoms in patients with CNS cancers. METHODS: We used two open source frameworks introduced by Apple to enable development of iOS operating system apps for medical research and personal care -- ResearchKit and CareKit -- to develop a mobile app framework for the CNS cancer context. Existing validated symptom reporting instruments, qualitative interviews, and evidence-based symptom management tools were used in the framework. RESULTS: We developed the My STORI mobile app to center on the experience of brain and spine cancer patients: patients and their family members can assess daily symptoms and impact and record any actions that were taken to mitigate them. Summaries of how these measures have evolved over time can be displayed in a series of plots and compiled into reports shareable with the care team. The app is based on research instruments that reflect the knowledge accumulated through years of clinical research in neuro-oncology. CONCLUSIONS: Mobile applications have the potential to promote self care, facilitate symptom management, as well as facilitate intuitive, frequent, and convenient collection of invaluable clinical-outcome research data. The My STORI app is an innovation in patient care inspired and guided by years of outcomes research, and tells an important aspect of the story of brain and spine cancer patients.

INNV-42. COST-EFFECTIVENESS OF INTRAOPERATIVE MRI IN THE TREATMENT OF HIGH-GRADE GLIOMAS

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OBJECT: High-grade gliomas (HGGs) have poor survival and high treatment costs. Intraoperative MRI (ioMRI) improves gross-total resection (GTR) rates and prolongs progression-free survival (PFS) in HGGs, but questions regarding its cost-effectiveness persist. To date, no clinical decision analysis models assessing ioMRI in the treatment of HGG exist. METHODS: An integrated 5-state microsimulation model was constructed to follow patients with HGG. Patients treated with ioMRI were compared to those without ioMRI from initial resection/debulking until death. Following surgery and treatment of complications, patients existed in one of 3 health states: PFS, progressive disease, or dead. Patients with recurrence were offered up to two repeat resections. PFS, health utility values, probabilities, and costs were obtained from randomized-controlled trials whenever possible. Otherwise, national databases, registries, and non-randomized trials were used. Uncertainty in model inputs was assessed using deterministic and probabilistic sensitivity analyses. A healthcare perspective was taken for this analysis. A willingness to pay (WTP) threshold of $100,000/QALY gained was used to determine cost-efficacy. RESULTS: IoMRI yielded an incremental benefit of 0.18 QALYs (1.16 QALYs without ioMRI vs. 1.34 with) at an incremental cost of $13,447 ($157,000 without vs. $170,447 with) in microsimulation modeling, resulting in an incremental cost-effectiveness ratio (ICER) of $76,442 per QALY. Given our parameter distributions, probabilistic sensitivity analysis demonstrated that ioMRI had a 99.5% chance of cost-effectiveness at a WTP threshold of $100,000/QALY. CONCLUSION: Intra-operative MRI is likely a cost-effective modality in the treatment of HGGs.

INNV-43. NUCLEIC ACID ADEQUACY FROM ARCHIVED FORMALIN-FIXED PARAFFIN EMBEDDED (FFPE) TUMOR TISSUE FOR NEXT-GENERATION SEQUENCING (NGS) IN NATIONAL CANCER INSTITUTE (NCI)- NATURAL HISTORY STUDY (NHS) OF PRIMARY CNS TUMOR

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BACKGROUND: A unified data collection system for interrogating the clinical trajectory of patients and their molecular pathology is of significant diagnostic, research, and therapeutic importance. The NCI-NHS follows primary CNS tumor patients throughout their disease. A targeted NGS panel was developed for clinical screening of mutations in 56 genes, 21 copy number alterations, and 25 gene fusion pairs using archived FFPE tissue. Due to the nature of study, available specimens range in age and source, posing a considerable challenge to obtaining an adequate quality and quantity of nucleic acid for genomic analysis. METHODS: Tumor blocks or unstained slides were received for macrodissection of neuro-pathologist scored tumor tissue. DNA and RNA were extracted, using the Qiagen AllPrep FFPE Kit on a semi-automated QIAcube instrument, and sequenced on Ion Torrent NGS platform. RESULTS: Tissue material from 227 patients was submitted, 17(8%) were rejected for low tumor content (<10%) and/or insufficient tissue. In total, 221 specimens were processed from 210 patients. Specimen ages as follows: < 5 years old, 69%; 5–9 years, 22%; ≥10 years, 9%. DNA was successfully sequenced for 99.5% of these cases. RNA sequencing success rate was 85% overall, with 91% for specimens <5 years old, 73% for 5–9 years, and 53% for ≥ 10 years. There was no significant difference between NIH and outside cases. Clinically significant molecular findings were reported in 81% of the cases across 18 diagnoses. High grade tumors comprised 78% of those calls. CONCLUSION: Robust variant calling for a variety of clinical specimens is critical in genetic medicine. Using our protocol, archived FFPE tumor specimen provided high quality NGS results, regardless of originating institution. Additionally, RNA from tissue over 5 years of age had a 71% sequencing success rate. This study confirms the feasibility of using archived materials for molecular analysis with analyzable results.

INNV-44. GRAM POSITIVE COLONIZATION OF THE OMMAYA RESERVOIR; TREATMENT RECOMMENDATIONS WITH CLINICAL EXAMPLES.

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BACKGROUND: Infectious complications of Ommaya reservoirs occur in 3–15% of patients and can lead to removal of the device and other complications. Removal of the device is suboptimal as intrathecal chemotherapy

is then delayed or discontinued. Gram positive skin flora, staphylococci and *Propionibacterium acnes*, are the most common colonizers of the reservoir. Understanding how to treat these bacteria can prevent poor outcomes. Both staphylococci and *P. acnes* are biofilm-forming bacteria. In implants colonized with *P. acnes*, a modeling study showed single agent rifampin to have a 36% cure rate. Despite good biofilm penetration, Rifampin's bactericidal activity is not as effective as when combined with other agents. When combined with daptomycin, the cure rate increased to 63%. METHODS: We evaluate two cases of *P. acnes* colonization in the Ommaya reservoir that illustrate effective treatment. RESULTS: Case-1: Ommaya placed in September, 2016 for intrathecal treatment of malignant meningitis with regularly occurring taps. In February 2018, CSF cultures revealed colonization with *P. acnes*. Patient was asymptomatic with CSF WBC of 0. Patient was treated with IV daptomycin 6mg/kg via PICC line daily for 14 days plus oral rifampin 300mg daily for 14 days. CSF cultures were negative after 7 days of antibiotics. Case-2: Ommaya placed in December, 2017 for intrathecal treatment of malignant meningitis. In February 2018, CSF cultures were positive for *P. acnes* with elevated CSF WBC and headaches. Patient refused IV antibiotic therapy and was treated only with oral rifampin 300mg daily for 14 days. CSF cultures were negative after 11 days of antibiotics. CSF WBC returned to normal limits; with resolution of headache. CONCLUSION: We recommend monitoring for colonization of insidious skin flora in patients on long term intrathecal chemotherapy using CSF cultures with every tap. Gram positive colonization can be detected early and treated effectively.

INNV-45. RADIANS: A MULTIDISCIPLINARY CENTRAL NERVOUS SYSTEM CLINIC MODEL FOR RADIATION ONCOLOGY AND NEUROSURGERY PRACTICE

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INTRODUCTION: Radiation therapy for central nervous system disease commonly involves clinical collaboration between the disciplines of Radiation Oncology and Neurological Surgery. Unfortunately such collaboration has been rarely implemented in the outpatient clinic setting. We describe our experience with a multidisciplinary clinic model featuring radiation oncology in tandem with neurosurgery in a community hospital setting. METHODS: In August 2016, we initiated the novel RADIANS (RADIation oncology And NeuroSurgery) clinic model at a community hospital, with the fundamental idea being to establish a weekly time where patients could receive input from both disciplines during a single clinic visit. Disease and treatment demographics were collected and analyzed. Patient satisfaction was assessed via survey questionnaire, where both radiation oncology and neurosurgery attendings were blinded to both the administration of and patient responses in surveys. RESULTS: The majority of patients were seen for metastatic disease of the brain and/or spine. Of patients receiving radiation, most received stereotactic radiosurgery (SRS)/SBRT rather than conventional fractionation. Survey responses revealed that 86% found that having a consultation with two physicians at the same time in the same room "was a totally new experience". All responders found that having two physicians at the same time was "a better way to be evaluated than simply having two separate appointments with these two physicians", while describing the dynamics of the evaluation process as being shared equally between the radiation oncologist and neurosurgeon. CONCLUSIONS: The majority of radiation therapy provided through the RADIANS clinic model has been SRS/SBRT rather than conventional fractionation. The model is popular with patients, most of whom present with brain and/or spine metastatic disease. Continued meticulous collection and analysis of outcomes will be needed to rigorously evaluate the long-term impact of RADIANS.

INNV-46. EVALUATING THE DECISIONS OF GLIOMA PATIENTS REGARDING CLINICAL TRIAL PARTICIPATION, A RETROSPECTIVE SINGLE PROVIDER STUDY

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BACKGROUND: Clinical trial accrual is vital to advancing care. A single study elucidated demographic data correlating with glioma patients' clinical trial enrollment. However, it did not investigate the underlying decision-making process, a key point to understanding why patients decline clinical trials. METHODS: All notes for glioma patients seen by a single neuro-oncologist from July 2010-May 2017 were examined. When a trial was declined, the patient's reasoning was recorded along with the following: diagnosis, KPS, extent of resection, age, gender, race, marital status, religion, trial offered at initial visit vs subsequent, and distance from trial site. RESULTS: Of 92 patients offered a clinical trial, 57 (65%) accepted and 31 (35%) declined. Patients with glioblastoma (GBM) were significantly more likely to accept a trial (44 (72%) vs. 13 (48%), P=0.03). Adjusting for gender and travel distance, GBM was the only significant predictor of clinical trial acceptance, having 3.18 higher odds (95% CI: 1.17, 8.61, P=0.02). Reasons cited for non-participation included: travel distance (39%), lack of interest (39%), visit frequency (16%), and fear of randomization (6%). CONCLUSIONS: This study clarified for the first-time individual glioma patient rationale for non-participation and potential areas for improving enrollment. Allowing off-site treatment centers or telemedicine visits may entice rural patients to participate. Visit frequency should be carefully considered and minimized whenever possible. Further prospective study of rationale for non-participation may improve enrollment over time.

INNV-47. TREATMENT ALLOCATION AMONG PATIENTS EVALUATED IN A MULTIDISCIPLINARY RADIATION ONCOLOGY AND NEUROSURGERY CENTRAL NERVOUS SYSTEM CLINIC

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INTRODUCTION: Treatment allocation for central nervous system disease has rarely been examined in a collaborative fashion. We describe our experience with RADIANS (RADIation oncology And NeuroSurgery), a novel multidisciplinary clinic model featuring radiation oncology in tandem with neurosurgery in a community hospital setting. METHODS: RADIANS patients were assessed to determine treatment allocation: surgery alone, radiation therapy (RT) alone, surgery with RT, or observation. Patient satisfaction was assessed via survey questionnaire. RESULTS: Forty-two patients have been seen since RADIANS inception. Median age was 65; median patient distance from RADIANS was 42.7 miles (mean=62.6; range 0.7–285); half of patients travelled more than 50 miles to receive care. Patients were most commonly treated with surgery and RT (33.3%), followed by RT alone (28.6%), observation (26.2%), and least commonly surgery alone (11.9%). More than three-fourths of patients were seen for metastatic disease of the brain and/or spine; 75% of patients delineated to RT received stereotactic body RT (SBRT). Lesions were nearly equally distributed between the brain (22 patients) and spine (20 patients). A majority of survey responders felt comfortable receiving two separate bills rather than a single bill for their RADIANS visit; all responders would recommend RADIANS for a friend/relative with a newly diagnosed spine or brain tumor. The average overall satisfaction on a 0 (not satisfied) to 5 (very satisfied) scale was 4.8. CONCLUSIONS: The RADIANS clinic model has proved viable in a community setting; half of patients travel a great distance to receive care. The most common treatment modality has involved SBRT with or without surgery. A large majority of patients are referred with metastatic disease. Thorough study will be needed to optimally evaluate the long-term impact of RADIANS on patient education and quality of care.

INNV-48. TUMOR TREATING FIELDS UTILIZATION IN A GLIOBLASTOMA PATIENT WITH A PREEXISTING CARDIAC PACEMAKER: THE FIRST REPORTED CASE

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INTRODUCTION: Tumor treating fields (TTF) have become an important, evidence-based modality in the treatment of glioblastoma (GBM). In patients requiring cardiac pacemakers, TTF therapy is complicated by theoretical concerns regarding a possible electrical interaction between the devices. The authors report a GBM patient with an indwelling cardiac pacemaker who underwent successful TTF application. METHODS: A 57-year-old man with past medical history of sick sinus syndrome requiring cardiac pacemaker implantation suffered an acute neurologic change and was found on imaging to have a left parieto-occipital lesion, which following his second surgery was found to be GBM. After completion of guideline-concordant chemoradiation, he chose to undergo TTF therapy. Because of the absence of cardiac symptoms and the theoretical risk of far-field sensing by the pacemaker of the TTF device, potentially resulting in pacemaker inhibition, the pacemaker was turned off prior to receiving TTF. RESULTS: Following TTF implementation, the patient responded well; he remains alive more than 25 months following his GBM diagnosis, exceeding the median 20.9 month survival of the recently completed phase III TTF randomized clinical trial for newly diagnosed GBM. Furthermore, he has exhibited neither cardiac morbidity nor adverse scalp reactions to TTF therapy. CONCLUSIONS: The first reported case of successful TTF administration in a GBM patient with a previously implanted cardiac pacemaker may allay the concerns of neuro-oncologists, cardiologists, radiation oncologists, and all certified TTF prescribers regarding the applicability of TTF in suitable candidates with preexisting cardiac pacemakers. This case indicates that TTF therapy is efficacious in patients with indwelling MRI-conditional cardiac pacemakers

turned to the off position, and that physical removal of the pacemaker is not necessary prior to starting TTF.

MENINGIOMA

MNGI-01. SURGICAL RESECTION AND ORBITAL RECONSTRUCTION IN SPHENO-ORBITAL MENINGIOMAS

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OBJECTIVE: To describe surgical technique and outcomes of surgical resection of intracranial, intra-orbital and intra-osseous disease in sphenoorbital meningiomas (SOMs) with periorbital and bony orbit reconstruction. METHODS: We performed a single institution retrospective review of consecutive cases between 2005 and 2016. Meningioma pathology records of 1007 patients revealed 125 sphenoid meningiomas of which 32 patients met eligibility criteria for SOMs. Clinical findings, radiographic features, operative technique, outcomes and follow-up data are presented. RESULTS: Of the 32 patients 20 met inclusion criteria. Mean follow-up duration was 69 months. The mean age of patients was 55 (range 32–82) with a female predominance (16F:4M). 4 (20%) had vascular involvement and 12 (60%) cavernous sinus infiltration. 9 (45%) tumors extended medially to the foramen rotundum and ovale. 8 (40%) patients did not require a bony orbital reconstruction, 11 (55%) had a split calvarial bone graft and 1 (5%) patient had a temporalis muscle graft. In 19 (95%) patients, pericranium was used for reconstruction of the peri-orbita and dura. 8 (40%) patients had complete clearance of their orbital disease. All patients presented with proptosis and in the majority this was corrected. 17 (85%) patients had WHO grade I disease with the other 3 (15%) WHO grade II disease. Of the 13 patients who had visual impairment, 9 patients had improved vision postoperatively, including 3 patients whose vision returned to normal. CONCLUSION: The management of SOM remains challenging for both resection and reconstruction. The goals of surgery entail recovery of compromised CN function and long-term tumor control. A multidisciplinary approach allows for safe resection with improvement in cosmesis. The benefits of orbital reconstruction are: structural, cosmetic, and provide landmarks for postoperative imaging.

MNGI-02. SUBCENTIMETER MENINGIOMAS – IS IT OKAY TO IGNORE?

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BACKGROUND: Meningiomas are among the most common incidental neuroimaging findings. Although most are indolent, a subgroup of meningiomas will eventually warrant clinical intervention. While larger diameters (e.g. ≥2.5cm) predict more aggressive behavior, there is a paucity of data regarding how much surveillance smaller meningiomas require if any. PURPOSE: To assess radiographic features of subcentimeter meningiomas that may predict future progression. METHODS: We included asymptomatic treatment naïve patients with subcentimeter probable meningiomas who had ≥6 months of MRI follow-up. Progression was defined as reaching the 1cm threshold or becoming symptomatic. Imaging characteristics such as presence of calcification, perilesional edema, T2-weighted intensity were assessed in relationship to tendency to progress via log-rank. RESULTS: Thirty-nine patients (mean age 62y, 34F) were identified, who had a total follow-up of 251 years. Twenty-four patients (60%) remained subcentimeter by the end of follow-up. Median time to reach 1cm was 3.0 years for the remaining 15 patients. Only 2 enlarged to 1.5cm, a 69-year-old female who reached this size after 4.5 years and a plateaud on subsequent scans. The other was a 71-year-old male whose tumor doubled about every 3 years, leading to resection after 7 years at 33mm. He harbored a grade 2 meningioma. None of the patients became symptomatic during the follow-up period. None of the patient's lesions had peritumoral edema. T2W intensity did not predict progression. In contrast, only 1 patient with a calcified meningioma reached 1cm. Calcification predicted slower progression (12.1 vs 6.8 years, log-rank p=0.03). CONCLUSION: Our data corroborates that most subcentimeter meningiomas grow extremely slowly. A single scan confirming calcification in the tumor may further support this growth pattern. However, repeated imaging seems necessary to identify exponential growth pattern, which is associated with higher grade meningiomas that can still present in this population.

MNGI-03. PATIENT PARAMETERS ASSOCIATED WITH TUMOR GROWTH IN INCIDENTAL MENINGIOMA

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BACKGROUND: Meningiomas are the most common primary intracranial tumors. Obesity or nulliparity are established risk factors for the development of these tumors. While radiographic tumor characteristics have been shown to be predictive of future tumor growth as demonstrated by the Asan Intracranial Meningioma Scoring System (AIMSS) [1], the relation of patient characteristics with tumor growth has been largely unexplored. The present study aims to evaluate a set of patient characteristics for predicting tumor growth and to assess whether these may provide information in addition to radiographic data for the prediction of progression free survival (PFS). METHODS: Ninety-two treatment-naïve patients with incidental meningiomas (mean±SD age 62 ± 14y; 73F) were enrolled with at least 2 contrast-enhanced MRIs acquired in a state ≥0.5y apart (mean: 4.3 MRIs/patient; mean follow-up time 4.5 ± 3.8y). Age, height, BMI, sex were recorded for all patient, and parity available for 32 patients. Two-dimensional tumor growth rate(TGR, mm^2/mo) calculated for all patients, while the AIMSS score was recorded for 81 patients. PFS was established as per the RANO criteria, defining progression as ≥25% increase in size. RESULTS: Age had a weak positive (r=0.21), while height had a weak inverse correlation (r=-0.19) with TGR (p CONCLUSION: Our preliminary data suggests that population data such as age, sex, height and BMI may have a value independent of radiographic characteristics to predict progression in incidental meningioma. Of these parameters, BMI is a modifiable risk factor. Weight loss could be evaluated as a potential intervention to slow down meningioma growth. [1] Lee, EJ, et al. *Journal of neurosurgery* 127.5 (2017): 971–980.

MNGI-04. A PROPOSED IMAGING-BASED NOMENCLATURE SYSTEM FOR MENINGIOMAS

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INTRODUCTION: Since Cushing and Eisenhardt first classified tumors of the meninges in their seminal 1938 work, *Meningiomas*, the general schema by which these tumors are described has changed little. However, over time several sub-classifications have been developed based on more granular anatomic localization, surgical approach, and treatment outcomes. This has led to significant variability in reporting meningioma nomenclature in the clinical and research settings. In the era of modern medicine, MR images are routinely acquired in meningioma patients at diagnosis or preoperatively. Here we evaluated whether a standardized meningioma nomenclature schema based on anatomic MR imaging could be developed and adopted in practice. METHODS: We reviewed our institutional database of > 2400 meningioma patients with pretreatment MR imaging and categorized the tumors by location based on gadolinium enhanced T1 weighted images. Whenever possible, multiplanar reformations from 3D spoiled gradient recalled acquisition in steady state (SPGR) images were utilized to precisely localize tumor. RESULTS: Meningiomas were classified into convexity, falx, falcotentorial, tentorial, parasagittal, orbital roof, olfactory groove, planum sphenoidale, tuberculum sellae, sphenoid wing, clinoidal, anterior petrous face, internal auditory canal, posterior petrous face, cavernous-gassero-petrosal, cerebellar convexity, clival, petroclival, foramen magnum, and intraventricular (atrial, third, and fourth ventricular) groups. For cases where a meningioma involved multiple locations, nomenclature was assigned based on an anterior-to-posterior and lateral-to-medial structure. CONCLUSION: Using Cushing's original nomenclature as a framework, and taking into account newer sub-classifications, we propose an MR imaging-based anatomic nomenclature for meningiomas. Implementing a standardized nomenclature schema for meningioma has the potential to harmonize collaborative research efforts and improve clinical communication between neuro-oncologists, neurosurgeons, radiation-oncologists, and radiologists.

MNGI-05. DEVELOPMENT AND VALIDATION OF A DNA METHYLOME-BASED PREDICTOR OF MENINGIOMA RECURRENCE AND MENINGIOMA RECURRENCE SCORE

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The greatest clinical challenge faced in meningioma is the inability to predict recurrence for individual patients, limiting the ability to select patients who would benefit from adjuvant radiation therapy to prevent recurrence. Therefore, in this multi-center cohort study, we utilized global epigenome DNA methylation profiles from human meningioma samples to generate and validate a methylome-based predictor of recurrence-free survival (RFS) and a meningioma recurrence score. Cox modeling of individual probes was used for feature selection in a training set (N=228 patients) which was then applied to two independent validation sets (N=54; N=140 patients). Gene-expression analysis was correlated to DNA methylation profiles using two published microarray datasets (GSE16581;GSE9438). Finally, penalized Cox modeling was used to generate a 5-year meningioma recurrence score based on a nomogram that integrated our validated methylome-based predictor with established clinical factors. The methlyome-based predictor was independently associated with RFS in each of the two validation sets, after adjusting for tumor grade and extent of resection (EOR; HR 4.0, 95%CI 1.4 – 11.5, P = 0.01 and HR 2.3, 95%CI 1.4 – 3.8, P=0.002). Using a 5-year RFS metric, the methylome-based predictor performed favourably compared to a grade-based predictor in both validation cohorts (ΔAUC =15%; ΔAUC=12%). Functional annotation of the included probes implicated the homeobox gene family. A nomogram constructed using the validated methylome-predictor with WHO grade and EOR demonstrated greater predictive performance than a nomogram using clinical factors alone (ΔAUC = 7.7%) and resulted in two different risk groups with distinct recurrence patterns (P <0.001). The methylome-based predictor and meningioma recurrence score developed and validated in this study provide important prognostic information not captured by established clinical factors and represent the first combined molecular and clinical prognostic tool that is individualized for patients with meningiomas, and hence an advance towards precision medicine in meningiomas

MNGI-06. MENINGIOMA METASTASES: INCIDENCE AND SCREENING IN 1203 PATIENTS

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OBJECTIVE: Extracranial meningioma metastases are rare, and patients with meningiomas are not routinely screened for systemic metastases. We report our experience with meningioma metastases and screening for metastases in select patients with recurrent meningiomas. METHODS: All patients undergoing surgical resection or stereotactic radiosurgery for primary or recurrent meningioma from 2009 to 2017 at a single center were retrospectively reviewed. Indications for metastasis screening were patients with recurrent meningioma after 2 prior resections and radiation therapy or symptoms concerning for metastasis. Screening was performed with CT chest/abdomen/pelvis, FDG PET-CT, or somatostatin receptor specific (DOTATATE) PET-CT. RESULTS: Of 1203 patients treated, 929 (77.2%) had WHO grade 1 meningioma by surgical pathology or imaging, 234 (19.5%) had grade 2 and 35 (2.9%) had grade 3 meningioma. A total of 298 meningiomas (24.8%) recurred, with a mean of 1.6 recurrences per patient. Screening for metastases was performed in 28 patients; one (3.6%) had a grade 1 tumor, 16 (57.1%) were grade 2, and 11 (39.3%) were grade 3. Five patients (17.9%) were screened because of systemic symptoms. Of the patients screened, 27 (96.4%) had recurrent meningioma, with a mean 3.2 recurrences. Ten patients (35.7%) had suspicious extracranial lesions by imaging. On biopsy, 8 were meningioma metastases, 1 was a non-meningioma malignancy, and 1 was lost to follow-up. Biopsy-confirmed metastases occurred in: liver (5), lung (3), mediastinum (1) and bone (1). The overall incidence of metastases was 0.67% (n=8). Incidence increased to 2% of WHO grade II tumors and 8.6% of grade 3. Using our indications, the number needed to screen to identify one patient with biopsy-confirmed malignancy was 3.1. CONCLUSIONS: Screening of patients with multiply recurrent meningioma or symptoms concerning for metastasis may identify extracranial metastases in a significant proportion of patients and can inform decision making for additional treatments.

MNGI-07. THE ANAPLASTIC MENINGIOMA INTERNATIONAL CONSORTIUM (AMICo) RETROSPECTIVE STUDY OF TREATMENT AND OUTCOME OF PATIENTS WITH ANAPLASTIC MENINGIOMAS

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Anaplastic meningiomas are rare, comprising only 1–2% of all meningiomas, and limited data on its natural history and response to treatment. An international retrospective study using 2016 Classification of the Tumours of the CNS was used to identify grade 3 meningiomas. Anonymised clinical and radiological data were collected in a uniform manner from 21 centres. RESULTS: 355 patients were identified to have an anaplastic meningioma. 57% cases arose *de novo* and 43% were progressed from lower grade; 20% directly from grade 1, 15% from grade 1 via grade 2 and 65% from grade 2. Female predominated among *de novo* and males among the progressive cases, with M/F ratios 0.8 and 1.4, respectively (p=0.02). *De novo* tumours were, on average, larger than progressive (51cc and 27cc, respectively; p=0.002). *De novo* cases predominated in sphenoid wing and ventricular locations, while progressive cases predominated in anterior fossa midline locations. The median survival of patient from the first diagnosis of anaplastic meningioma (index surgery) was 99 and 30 months for *de novo*and progressive cases, respectively (p<0.0001). Independent factors for survival were degree of resection (p=0.23) and administration of radiotherapy (p<0.001). Among the progressive anaplastic meningiomas, the longest survival was achieved in patients who received radiotherapy both before and after the index surgery. Those receiving radiotherapy only after lived longer than those who received radiotherapy before the index surgery. Those who received no radiotherapy had shortest survival. The difference in growth between surgery only and surgery and radiotherapy groups was significant (17.6%/month versus 5.3%/month; p = 0.038). Here in we present the largest cohort of patients with anaplastic meningiomas allowing detailed analysis of natural history and response to treatment in *de novo* and progressive anaplastic meningiomas.

MNGI-08. A RARE CASE OF INFANTILE PAPILLARY RHABDOID MENINGIOMA

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Infantile meningioma is exceedingly rare, frequently larger and of higher-grade histology than older patients. We describe a case of papillary meningioma with rhabdoid features in a 6-month-old, previously healthy infant. During evaluation for accidental head trauma the patient was found to have a large heterogeneous mass in the right temporal parietal region with midline shift and uncal herniation on cranial Computed Tomography (CT). Magnetic resonance imaging (MRI) of the brain revealed a large extra axial middle cranial fossa lesion with involvement of sphenoid and extraconal space of right orbit. There was no metastatic disease on MRI spine, CT chest, abdomen and CSF studies. A diagnostic cerebral angiogram showed a hyperplastic right middle meningeal artery (MMA) supplying the tumor. Skull base near total resection of tumor was performed after embolization of the right MMA pedicle artery and right ophthalmic artery recurrent branch. Pathology was consistent with papillary meningioma with focal rhabdoid features. Comprehensive genomic profile was positive for Neurofibroma-

tosis (NF) type II. MRI obtained 4 weeks after initial resection revealed local progression. Gross total resection(GTR) of residual tumor was performed. In view of rapid progression, chemotherapy was initiated 12 weeks from diagnosis, as per Dana Farber Protocol 02-294 for ATRT (Atypical Teratoid Rhabdoid Tumor). Therapy related complications included – febrile neutropenia, *Candida rugosa* fungemia, loss of vision in right eye and right ear sensorineural hearing loss. The patient continues to be in remission 1 year after completion of his therapy. There are no established guidelines for the management of pediatric rhabdoid meningiomas. Multiagent chemotherapy as per DFCI ATRT 02-294 along with GTR should be considered as a potential treatment option for papillary rhabdoid meningioma. Radiation therapy should be excluded in younger patients due to risk of neuro-cognitive sequelae. Patients with meningioma associated with NF II need to have lifelong follow up.

MNGI-09. FERTILITY TREATMENT AND MENINGIOMA INCIDENCE

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OBJECTIVE: Meningiomas are more common in females and 70–80% express the progesterone receptor. They have been reported after gender reassignment therapy, suggesting that high-dose exogenous estrogen/progesterone exposure, such as occurs during fertility treatments, may increase the risk of developing a meningioma. The goal of this study was to report the incidence of prior fertility treatment in a consecutive series of female patients presenting with meningioma. METHODS: A retrospective review of patients presenting with meningioma from 2015–2018 was conducted. Female patients with prior fertility treatments were compared to those who did not receive fertility treatment using standard statistical methods. RESULTS: Of 206 female patients with meningioma, 26 (12.6%) had a history of fertility treatments. Patients underwent various forms of assisted reproductive technology including: in vitro fertilization (50.0%), clomiphene with or without intrauterine insemination (34.6%), and unspecified (3.8%). One patient (3.8%) received supplemental progesterone during her treatment. The most common presenting symptoms were incidental (57.7%) and headache (26.9%). Median follow up was 1.8 years. Tumors were WHO grade I (78.6%) or grade II (21.4%). Patients who underwent fertility treatments presented at significantly younger mean age compared to those who had not (51.8 vs 57.3 yrs, p = 0.0135, 2-tailed T-test) and were more likely to have multifocal (OR: 4.5, 95% CI: 1.4–14.8, p = 0.0196) and non-skull base meningiomas (OR: 4.4, 95% CI: 1.7–11.4, p = 0.0012). CONCLUSIONS: A history of fertility treatment is common in female patients presenting with meningioma. Patients with meningioma and a history of fertility treatment were more likely to present at a younger age and have multifocal and non-skull base tumors. These findings stress the importance of assessing for prior estrogen/progesterone exposure in patients presenting with meningioma. Future large prospective series and laboratory investigations are needed to determine the impact of fertility treatment on meningioma development.

MNGI-10. PREDICTORS OF EARLY PROGRESSION OF SURGICALLY TREATED ATYPICAL MENINGIOMAS

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BACKGROUND: Clinical behaviour of atypical meningiomas is not uniform. While, as a group, they exhibit a high recurrence rate, some pursue a more benign course, whereas others progress early. We aim to investigate the imaging and pathological factors that predict risk of early tumour progression and to determine whether early progression is related to outcome. METHODS: Adult patients with WHO grade II meningioma treated in 3 regional referral centres between 2007 and 2014 were included. MRI and pathology characteristics were assessed. Gross total resection (GTR) was defined as Simpson 1–3. Recurrence was classified into early and late (≤ 24 months vs. > 24 months). RESULTS: Among the 220 cases thirty-seven (16.8%) patients progressed within 24 months of operation. Independent predictors of early progression were subtotal resection (STR) (p=0.005), parafalcine/parasagittal location (p=0.015), peritumoural oedema (p=0.027) and mitotic index (MI) > 7 (p=0.007). Adjuvant radiotherapy was negatively associated with early recurrence (p=0.046). Thirty-two per cent of patients with residual tumour and 26% after GTR received adjuvant radiotherapy. There was a significantly lower proportion of favourable outcomes at last follow-up (mRS 0–1) in patients with early recurrence (p=0.001). CONCLUSIONS: Atypical meningiomas are a heterogeneous group of tumours with 16.8% patients having recurrence within 24 months of surgery. Residual tumour, parafalcine/parasagittal location, peritumoural oedema and a MI > 7 were all independently associated with early recurrence. As administration of adjuvant radiotherapy was not protocolised in this cohort any conclusions about benefits of irradiation of WHO grade II meningiomas should be viewed with caution. Patients with early recurrence had worse neurological outcome. While histological and imaging characteristics provide some prognostic value further molecular characterisation of atypical meningiomas is warranted to aid clinical decision making.

MNGI-11. LONGITUDINAL GENOMIC ANALYSIS OF SPORADIC MENINGIOMAS WITH MULTIPLE RECURRENCES

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Recently, genomic patterns and methylation profiles of meningiomas were described as prognostic factors for aggressiveness and recurrence (e.g. TERT, SMO and AKT1 mutations). However, the particular molecular profiles of recurrence and malignant progression of specific meningioma subtypes remain undefined. In this regard, longitudinal molecular studies are very interesting. In this study, we characterized somatic mutations in a cohort of sporadic meningioma patients. We included 9 patients with multiple recurrences and up to 11 subsequent resections of sporadic meningiomas. We performed a comparative whole-exome sequencing (WES). Moreover, we used the whole-exome data to predict the HLA MHC class I peptides of the subsequent tumor samples in silico. Our study included patients with follow-up times accounted for up to 26 years (average: 12.6 years, range 3–26). We detected a TRAF7 mutation (c.1189G>C) in only 2 of 6 recurrent tumor samples of a female patient with a malignant progression from a WHO I to a WHO grade II meningioma. In a male patient with 9 analyzed meningioma tumor samples, recurrent somatic PLEKHG5, AGBL1, ALK, RICTOR, DEPDC5 mutations were detected. This individual pattern remained constantly evident under different treatments, even after systemic treatment with the mTOR inhibitor everolimus. Moreover, unique mutations in HLA-DQA1, OR14J1, PRH1 genes occurred after mTOR inhibition in this male patient. Furthermore, we identified somatic mutations in the tumor suppressor candidate NBPF1 in 7 of 9 patients. HLA allele types were predicted in silico, with A*02:01, B*57:01, C*03:03, C07:01 and B51:01 being most frequent. Neither ubiquitously shared somatic mutations nor shared MHC class I peptides were identified among this cohort. Taken together, our study outlines a heterogeneous molecular profile in this analysis. Since longitudinal samples in sporadic meningioma are rather rare, the ICOM consortium would be an ideal frame for studying the molecular profile of recurrence in meningioma in larger cohorts.

MNGI-12. EXPRESSION OF PROGRAMMED CELL DEATH LIGAND-1 (PD-L1) IN MENINGIOMA: CLINICAL UTILITY FOR PREDICTION OF TUMOR RECURRENCE AND ASSOCIATION WITH HYPOXIC RESPONSE AND NFKB2 ACTIVATION

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Tumor recurrence is one of the most important clinical challenges in the management of meningioma patients. Prognostic significance of PD-L1 as a driver for immunosuppressive response and predictor for tumor growth has been demonstrated in several malignancies. We studied the prognostic role of PD-L1 expression for tumor recurrence in meningioma and explored underlying activation mechanisms. We analyzed a total of 93 meningioma cases diagnosed between 1998 and 2016 at University Health Network: F/M ratio 58/35; WHO grades I (43), II (42), III (9) with 47% recurrence rate and median follow up 6.97 years. Immunohistochemical (IHC) analysis on tumor sections showed PD-L1 expression in 33 (35%) cases with distinctive patchy distribution. Univariate and multivariate analyses confirmed that PD-L1 expression is an independent prognostic marker for recurrence free survival(RFS) in meningioma patients after adjusting for extent of resection, WHO grade, and maximum tumor diameter ($p<0.0001$). Additionally, we performed Gen Set Enriched Analysis (GSEA) on RNA seq data from 88 meningiomas using HUVEC hypoxia Dataset GSE89831 as reference to calculate hypoxia levels. Our results indicated that hypoxic meningiomas have significantly elevated PD-L1 expression. Furthermore, we investigated expression of PD-L1 in 3 different meningioma cell lines under normoxic and hypoxic conditions by real-time PCR. We found that in addition to the expected HIF1a target genes, PD-L1 mRNA level increased when exposed to hypoxic condition. Analysis of RNAseq data from two GEO meningioma studies demonstrated prominent NFKB2 activation associated with PD-L1 mRNA expression. IHC analysis confirmed expression of NFKB2 protein in 26 (30%) cases, which correlated with PD-L1 expression. Our data strongly suggest the clinical utility of PD-L1 expression for prediction of tumor recurrence and a potential link between hypoxia and anti-cancer immunity in meningioma patients. These results also provide a rationale for a potential therapeutic role for PD-L1 inhibitors in clinically aggressive meningioma.

MNGI-13. A DNA METHYLATION-BASED CLASSIFIER FOR ASSESSMENT OF RISK OF RECURRENCE IN MENINGIOMA

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The World Health Organization (WHO) classification of brain tumors comprises 15 histological subtypes of meningioma, allotted to three WHO grades. While the current classification and grading approach is of prognostic value, it harbors shortcomings due to the necessarily subjective evaluation of histological criteria and potential sampling bias. In order to devise a molecular approach to meningioma classification, we previously identified six distinct Methylation Classes (MCs) of meningioma by DNA methylation profiling. These six MCs are termed MC benign-1, -2, -3, MC intermediate A, B, and MC malignant. Each MC shows typical mutational, cytogenetic, and gene expression patterns. Importantly, MCs are superior to WHO grading in predicting the risk of recurrence for individual patients. In order to translate these findings into diagnostic practice, we developed a meningioma classifier, assigning novel diagnostic samples to the respective MC of the reference cohort. This classifier is now being used on diagnostic cases for 2 years at the Dept. of Neuropathology Heidelberg and has recently been made available for external users through molecularneuropathology.org, alongside the brain tumor entity classifier. Over 200 diagnostic cases have been evaluated so far. Integrating histology, classifier results, and copy-number alterations provides a more robust basis for grading and the subsequent clinical decision whether to administer radiotherapy or not. In addition, the association of certain subgroups with druggable mutations informs about the potential to identify specific targets by additional sequencing. Collectively, this tool opens the opportunity for an integrated classification of meningioma samples in routine diagnostics.

MNGI-14. LOSS OF HISTONE H3K27me3 IDENTIFIES A SUBSET OF MENINGIOMAS WITH INCREASED RISK OF RECURRENCE

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Epigenetic patterns on the level of DNA methylation have already shown to separate clinically relevant subgroups of meningiomas. Based on a reference set (Sahm et al., Lancet Oncol 2017), an epigenetic meningioma classifier employing DNA methylation patterns is made available through molecularneurpathology.org. We now set out to identify prognostic implications of epigenetic modification on the proteome level, particularly modifications of histones. First focus was on H3K27 trimethylation (H3K27me3). H3K27me3 was assessed by immunohistochemistry on 232 meningiomas. In 194 cases, trimethylation was detected in tumor cells. In 25 cases, staining was limited to vessels while all tumor cells were negative. Finally, 13 cases yielded equivocal staining patterns. Reduced abundance of H3K27me3 in cases with staining limited to vessels was confirmed by mass spectrometry on a subset of cases. Lack of staining for H3K27me3 in all tumor cells was significantly associated with more rapid progression ($p=0.009$). In line, H3K27me3 negative cases were associated with a DNA methylation pattern of the more aggressive types among the recently introduced DNA methylation groups. Also, NF2 and SUFU mutations were enriched among cases with lack of H3K27me3 in tumor cells ($p<0.0001$ and $p=0.029$, respectively). H3K27me3 staining pattern added significant prognostic insight in WHO grade II cases and in the compound subset of WHO grade I and II cases ($p=0.04$ and $p=0.007$, respectively). However, it did not further stratify within WHO grade III cases. Collectively, this data indicate that epigenetic modifications beyond DNA methylation are involved in the aggressiveness of meningioma. It also suggests that H3K27me3 immunohistochemistry might be a useful adjunct in meningioma diagnostics, particularly for cases with WHO grade II histology or at the borderline between WHO grade I and II. Ongoing studies evaluate the role of histone marks other than H3K27me3 and consequences on the proteomic composition of meningioma cells by high-throughput mass spectrometry.

MNGI-15. RARE PRESENTATION OF EXTRACRANIAL PARAVERTEBRAL MENINGIOMA IN A CHILD WITH NEUROFIBROMATOSIS TYPE 2: A CASE REPORT

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Meningioma is a rare intracranial tumor of children and adolescents. Meningioma occurring outside the central nervous system is even rarer. We describe an 11- year old child who presented with extracranial paravertebral skull base meningioma and left vestibular schwannoma with an underlying neurofibromatosis type 2 (NF2). The patient presented with hearing loss, painless left neck mass and right sided horner syndrome. A CT scan and MRI revealed a skull base tumor in the region of the left carotid body encasing the vessels, opacifying the left middle ear cavity, extending intracranially through the left jugular foramen along with regional lymphadenopathy and left vestibular schwannoma. MRI spine identified Arnold-Chiari I malformation and two extramedullary enhanc-

ing lesions (1-2cms) at the level of thoracic spine. With a differential diagnosis of sarcoma and peripheral never sheath tumor with metastasis to spinal cord, partial excision of the skull-base tumor was performed along with suprahyoid neck dissection. Bone marrow examination to rule out metastasis was normal. Pathology confirmed the diagnosis of meningioma (meningothelial variant, WHO grade-1). Lymph nodes showed reactive hyperplasia. With this clinical presentation of hearing loss, extracranial meningioma, vestibular schwannoma and spinal tumour likely to be spinal meningioma or schwannoma, a diagnosis of NF2 was suspected. Next generation sequencing showed deletion of exon 4 in NF2 gene. On further screening, early changes of cataract were seen in the right eye. Surgery couldn't be done for treatment of meningioma due to the encasement of the vital structures. Therapy with bevacizumab and everolimus was started to improve hearing loss secondary to schwannoma and extracranial meningioma. This report highlights the uncommon presentation of NF-2 and extracranial meningioma. Extracranial meningioma must be considered in the differential diagnosis in a child presenting with tumor of the skull base, middle ear and neck.

MNGI-16. TREATMENT FOR TUBERCULUM SELLAE MENINGIOMAS: ENDONASAL ENDOSCOPIC TRANSSPHENOIDAL VERSUS TRANSCRANIAL SURGERY. A META-ANALYSIS OF COMPARATIVE COHORT STUDIES

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BACKGROUND: Tuberculum sellae meningiomas (TSMs) are a distinctive subgroup of meningiomas in the suprasellar space, accounting for 5%-10% of all intracranial meningiomas. Traditionally, these tumors have been excised through a variety of transcranial approaches (TCA). The endoscopic endonasal transsphenoidal approach (EETA) has been used recently to treat TSMs. A meta-analysis was performed to compare the complications and outcomes between EETA and TCA for TSMs with comparative cohort studies, in order to enhance our understanding of the current outcomes of EETA for TSMs before the techniques become more widely used. MATERIAL AND METHODS: A meta-analysis of studies that compared the endoscopic with the transcranial approach was conducted. Data related to post-operative complications, gross total resection (GTR), visual improvement and recurrence was pooled to compare and analyze the results of the two treatment approaches for TSMs. RESULTS: Five published reports of eligible studies involving 161 participants met the inclusion criteria. There was no statistically significant difference between ETSA group and TCA group regarding total complications [RR = 1.47, 95%CI (0.67, 3.24), p = 0.34], complications of non-CSF leak [RR = 0.74, 95%CI (0.38, 1.47), p = 0.40], visual improvement [RR = 1.22, 95%CI (0.78, 1.92) p = 0.38], GTR [RR = 1.10, 95%CI (0.68, 1.77), p = 0.70], recurrence rate [RR = 0.98, 95%CI (0.24, 3.98), p = 0.98]. The occurrence of CSF leak in ETSA group was significantly higher than in TCA group [RR = 5.20, 95%CI (1.62, 16.68), p = 0.006]. CONCLUSION: In this meta-analysis of comparative cohort studies, EETA is comparable with TCA in terms of GTR, post-operative recurrence rate, complications other than CSF leak and visual improvement. As closing techniques for the prevention of CSF leaks in endoscopic approaches are evolving, further studies are warranted to focus on the reduction of post-operative CSF leak.

MNGI-17. THE ROAM / EORTC 1308 INFORMATION STUDY RESULTS: HOW QUALITATIVE RESEARCH METHODS CAN OPTIMISE PATIENT RECRUITMENT FOR MENINGIOMA TRIALS

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BACKGROUND: ROAM/1308 is an international randomised controlled phase III trial comparing radiation to observation following complete surgical resection of atypical meningioma. We embedded a qualitative sub-study within ROAM/1308 with the aim of optimising patient recruitment. METHODS: Patients approached to participate in the ROAM trial and recruiting clinicians were enrolled into the qualitative sub-study in 11 UK sites. Audio-recorded recruitment consultation (n=30), and semi-structured interviews with clinicians (n=17) and patients (n=23), including decliners and consenters. Analysis of transcribed audio-recordings was informed by content and thematic analysis. Ethics approval was granted for the study. RESULTS: Analysis identified areas where communication was problematic. Giving patients their pathology results immediately before discussing ROAM left them overwhelmed and unable to absorb trial information. Clinicians presentation of the trial arms often lacked balance with a tendency to emphasise the positive aspects of active monitoring (i.e. no additional treatment) while the negative aspects of tumour recurrence were rarely discussed. Conversely the negative aspects of radiotherapy were emphasised whilst neglecting to discuss that radiotherapy may confer better disease control. Patients exhibited bias against radiotherapy citing concerns about side effects, and this perception was rarely challenged by recruiting clinicians. Several patients viewed the prospect of radiotherapy as illogical, in part, due to earlier conversations with neurosurgeons who indicated further treatment was unnecessary following resection. CONCLUSIONS: Embedded qualitative studies can address barriers to recruitment in meningioma trials. The patient information leaflet has been amended and a patient-facing video added to the trial website. Workshops and a webinar for healthcare professionals (surgeons, oncologists research nurses) to enhance communication about ROAM/1308 have been conducted. Subsequent recruitment consultations had a more balanced discussion and clinicians felt more confident approaching patients about ROAM/1308. Ongoing support will be provided to sites to assist them in implementing and maintaining changes in the recruitment consultation.

MNGI-18. RISK FACTORS FOR PRE- AND POSTOPERATIVE SEIZURES IN MENINGIOMA PATIENTS IDENTIFIED BY LOGISTIC REGRESSION ANALYSIS

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OBJECTIVE: To identify independent risk factors of pre- and postoperative seizures in meningioma patients. METHODS: This is a retrospective single-center analysis of consecutive patients that underwent resection of intracranial meningioma between 2004 and 2017. The impact of patient and tumor characteristics on pre- and postoperative seizures was assessed by univariate and binary logistic regression analysis. RESULTS: Among 729 included patients, pre- and postoperative seizures occurred in 18.9% and 10.0%, respectively. In the univariate analysis, female gender ($p = 0.013$), preoperative motor deficits ($p < 0.001$) and peritumoral edema ($p < 0.001$) were associated with preoperative seizures. Headache ($p < 0.001$), preoperative sensoric symptoms ($p < 0001$) and occipital tumor location ($p = 0.024$) were negatively correlated with preoperative seizures. In the multivariate analysis, preoperative motor deficits ($p < 0.001$, OR: 3.9, 95% CI: 2.6 5.9) and peritumoral edema ($p < 0.001$, OR: 3.5, 95% CI: 1.8 6.7) were independent risk factors of preoperative seizures. In the univariate analysis postoperative seizures were significantly associated with parietal tumor location ($p= 0.021$), WHO grades 2 and 3 ($p < 0.001$), presence of multiple meningiomas ($p = 0.034$), incomplete tumor resection (Simpson grade > 2, $p = 0.007$) and recurrent meningioma ($p = 0.001$). Incomplete resection ($p = 0.04$, OR: 2.6, 95% CI: 1.1 6.6) and recurrent meningioma ($p = 0.037$, OR: 2.6, 95% CI = 1.1 6.2) remained as independent risk factors for postoperative seizures in the multivariate analysis. CONCLUSIONS: Preoperative motor deficits and peritumoral edema are independent risk factors for preoperative seizures, while incomplete tumor resection and recurrent meningioma are independently associated with postoperative seizures.

MNGI-19. SURGICAL RESECTION OF SPHENO-ORBITAL MENINGIOMAS AND ORBITAL RECONSTRUCTION USING PATIENT SPECIFIC CAD/CAM IMPLANTS

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INTRODUCTION: Computer-aided design and manufacturing (CAD/CAM) implants are fabricated based on volumetric analysis of computed tomography (CT) scans and are routinely used for the reconstruction of orbital fractures. We present three cases of patients with spheno-orbital meningiomas that underwent tumor resection, orbital decompression and orbital reconstruction with patient specific porous titanium or acrylic implants in a single procedure. METHODS: The extent of bone resection of the spheno-orbital meningiomas was planned in a virtual three-dimensional (3D) environment using preoperative thin-layer CT data. The anatomy of the orbital wall in the resection area was reconstructed by superimposing the contralateral unaffected orbit and by using the information of the neighboring bony structures. The customized implants were designed in the desired size and shape using computer-aided manufacturing. Furthermore, a corresponding craniotomy template in the form of a frame was produced for precise craniotomy. RESULTS: All patients presented with a spheno-orbital meningioma and an exophthalmus. After osteoclastic craniotomy with the drilling template orbital decompression was performed. The cranioplasty implant fitted tightly in all three cases

and could be easily fixated with mini-plates and screws, although in one case a reoperation was necessary for additional resection, as well as drilling and repositioning of the implant. The postoperative CT scans showed an accurate reconstruction of the orbital wall. After surgery, exophthalmos was substantially reduced and a satisfying cosmetic result was achieved in all patients. CONCLUSIONS: The concept of preoperative 3D virtual treatment planning and single-step orbital reconstruction with CAD/CAM-implants after tumor resection involving the orbit is well feasible and can lead to good cosmetic results.

MNGI-20. LARGE PERITUMORAL EDEMA RELATIVE TO TUMOR VOLUME PREDICTS SECRETORY SUBTYPE IN MENINGIOMAS

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BACKGROUND: Secretory meningiomas represent a benign subtype of meningiomas (WHO I°), but show a high prevalence of serious perioperative adverse events. Preoperative identification of a secretory subtype could facilitate risk stratification, and selection of pre- and perioperative medical treatment. OBJECTIVE: To evaluate the relationship of preoperative MRI features and secretory subtypes in meningioma patients. METHODS: All meningioma patients with available preoperative MRI that underwent tumor resection between 2013 and 2018 were reviewed. Different imaging characteristics were collected (i.e. tumor surface, arachnoid plane, T2 intensity) as well as the volume of tumor and peritumoral edema (PTE), using a semiautomatic image-processing software. In addition, the edema index (EI) (ratio of PTE to tumor volume) was calculated and all factors were correlated with histological subtypes. Receiver operating characteristic (ROC) curve analysis was performed to identify cut-off EI values to predict histological subtypes. RESULTS: We identified 163 patients, whereof seven (4.3%) presented with secretory meningiomas. EI ($p < .005$), as well as PTE ($p < .05$) proved to be the only parameters significantly associated with secretory meningioma. In ROC curve analysis, EI was the most sensitive and specific parameter to predict a secretory subtype. The optimal cut-off value at > 4.39 provided a sensitivity of 85.71% and a specificity of 95.57%. CONCLUSION: EI can be used as a highly specific and sensitive parameter to predict a secretory meningioma subtype, providing a useful tool for improvement of pre- and perioperative medical management.

MNGI-21. OPTIMISING PATIENT SELECTION FOR ANTIEPILEPTIC DRUG THERAPY IN MENINGIOMA SURGERY

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BACKGROUND: Epilepsy is a major cause of morbidity and mortality in meningioma patients. The aims of this study were to determine which factors predispose meningioma patients to developing perioperative seizures and to understand the impact of antiepileptic drugs. METHODS: Patients treated for a histologically-confirmed intracranial meningioma at the authors institution between 2010 and 2015 were retrospectively examined. Clinical and imaging data were assessed. Multivariate analysis was performed using binary logistic regression. The effect of antiepileptic treatment was assessed using survival analysis. RESULTS: Two hundred and eighty-three patients met the selection criteria; seizures were present in 68 (24%) preoperatively and in 48 patients (17%) following surgery. Of the 68 with preoperative seizures, 19 continued to have them, whereas de-novo seizures arose postoperatively in 29 seizure-naïve patients. Risk factors of postoperative seizures were convexity location (OR=2.05 [95% CI=1.07–3.98], p=0.030), frontoparietal location (OR=4.42 [95% CI= 1.49–13.16], p=0.007) and preoperative seizures (OR=2.65 [95% CI=1.37–5.24], p=0.005). The two locations, in addition to the presence of midline shift on preoperative imaging (OR=4.15 [95% CI=1.54–11.24], p=0.005), were significantly correlated with postoperative seizures in seizure-naïve patients. Antiepileptic treatment in patients with those risk factors reduced the possibility of seizures at any time point within the 1st year postoperatively by approximately 40%, although this did not meet statistical significance. CONCLUSION: Prophylactic antiepileptic treatment might be warranted in seizure-naïve meningioma patients with 1 risk factor. High-quality randomised controlled trials are required to verify those factors and to define the role of antiepileptics in meningioma practice.

MNGI-22. A PROGNOSTIC INDEX TO PREDICT THE RISK OF ACTIVE MONITORING FAILURE FOR INCIDENTALLY-FOUND ASYMPTOMATIC MENINGIOMAS

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BACKGROUND: 30% of meningiomas are incidental findings. There is no consensus on the optimal management (active monitoring, surgery, radiosurgery). OBJECTIVE: Develop a prognostic index to identify patients at risk of active monitoring failure. METHODS: Active monitoring failure was defined as: new symptoms, MRI progression (absolute growth rate 2 cm3/year or absolute growth rate 1 cm3/year + relative growth rate 30%/year) or loss of treatment options. A prognostic model was developed using MRI and patient co-morbidity (Charlson-Index) in a retrospective cohort (2007–2015). RESULTS: 385 patients (403 meningiomas) were studied; mean age was 62.6 years (SD=12.0); 301 (78.2%) were female. Over a median of 36.0 months (range: 3–120), 1688 MRI were performed (mean=4 scans/patient). 44 (10.9%) meningiomas failed active monitoring. Median time to failure was 33.0 months (range: 5–102). Model parameters were based on statistical and clinical considerations and included: increasing tumour volume (HR=2.17 [95% CI=1.53–3.09], $p<0.001$), peritumoural signal change (HR=1.58 [95% CI=0.65–3.85], p=0.313), FLAIR/T2 hyperintense meningiomas (HR=10.6 [95% CI=5.39–21.0], $p<0.001$) and proximity to neurovascular structures (HR=1.38 [95% CI=0.74–2.56], p=0.314). Discriminatory power of the model was excellent (Harrells C statistic=0.89). Patients were stratified into low, medium and high-risk groups and rates of failed active monitoring at 5-years were 3%, 28% and 75% respectively. Low-risk patients had non-oedematous, small iso/hypointense meningiomas, distant from neurovascular structures. After 5-years of follow-up, the probability of failed active monitoring plateaued in all risk groups. Older patients with co-morbidities (Charlson-Index 6) were 15-times more likely to die than to receive intervention at 5-years following diagnosis, regardless of risk-group. CONCLUSION: Most meningiomas remain clinically and radiologically stable. Patients with Charlson-Index 6 do not require active monitoring. Low-risk patients require less frequent MRI monitoring. Follow-up beyond 5-years may not be required for all patients. Stratifying follow-up according to risk-group has the potential to reduce the cost of healthcare.

MNGI-23. PREOPERATIVE QUANTITATIVE IMAGING FEATURES ARE PROGNOSTIC FOR MENINGIOMA OUTCOMES

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OBJECTIVES: Quantitative radiologic and radiomic features can identify brain tumors at risk for poor outcomes. Here, we investigate prognostic models for meningioma grade, local failure (LF) and overall survival (OS) based on demographic, radiologic, radiomic and therapeutic data. METHODS: We developed a database that was enriched for high grade meningiomas from 219 patients who underwent surgery for 229 meningiomas from 1990 to 2015 who had comprehensive clinical, pathologic and radiologic information available for retrospective review. The median imaging follow up was 4.3 years, and there were 112 WHO grade I (49%), 93 grade II (41%) and 24 grade III (10%) meningiomas. Two neuro-radiologists independently annotated 17 radiologic features, and 154 radiomic features were extracted from preoperative post-contrast 3D SPGR MR images for each meningioma. Random forest models were trained using nested resampling, and the performance of each model was assessed by calculating feature importance, mean balanced accuracy (BA) and area under the curve (AUC). RESULTS: Models restricted to preoperative demographic information and quantitative imaging features had superior BA (0.60–0.67) and AUC (0.60–0.76) for LF or OS as compared to models based on meningioma grade and extent of resection (BA 0.65, AUC 0.64). Integrated models incorporating all available data provided the most accurate estimates of LF and OS (BA 0.67, AUC 0.76). Radiomic features alone or in combination with other variables showed moderate and marginal predictive value for grade (BA 0.63, AUC 0.72) and LF (BA 0.61, AUC 0.65), respectively. Among radiologic features, meningioma diffusion characteristics significantly strengthened prognostication of grade and LF (RR 25.6, P = 0.001). Recursive partitioning analysis identified tumor size, primary versus recurrent presentation, grade, sphericity, apparent diffusion coefficient, location, extent of resection and T2 signal as the most important features for LF. CONCLUSIONS: Mod-

els using clinical and quantitative imaging data can accurately predict meningioma outcomes.

MNGI-24. PREDICTORS OF VISUAL OUTCOMES IN SPHENO-ORBITAL MENINGIOMA SURGERY ENDORSE EARLY MAXIMUM SAFE SURGERY AND INTENSIVE FOLLOW-UP OF PATIENTS WITH MULTIPLE MENINGIOMA

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OBJECTIVE: Most Spheno-Orbital Meningioma (SOM) series include patients over a period of multiple decades, while surgical techniques have improved over the years. In addition, predictors of visual outcomes and progression free survival (PFS) have not yet been systemically assessed. The aim of this study was to assess predictors of visual outcomes and PFS in a recent SOM cohort. METHODS: Consecutive patients operated by a team of a neurosurgeon and orbital surgeon in the Leiden University Medical Center between June 2015-December 2017 were included. Pre- and postoperative visual acuity (Snellen chart), visual field deficit (Humphrey field analyser, in decibel [dB]), and relative proptosis (exophthalmometry) were compared with the Wilcoxon signed-rank test. Predictors of visual outcomes were assessed with linear regression analysis. Predictors of PFS (definition: need for reintervention) with the log-rank test. RESULTS: Eight patients presented with impaired visual acuity, which improved in 88% (preoperative: 0.8, postoperative: 1.1, p=0.012). All 16 patients presented with visual field deficits, which improved in 86% (preoperative: -8.4dB, postoperative: -3.8dB, p=0.008). Also all patients presented with proptosis, which improved in 86% (preoperative: 4.5mm, postoperative: 2.9mm, p=0.013) Strongest predictors for postoperative visual acuity, visual field deficits and persistent proptosis were preoperative visual acuity (p=0.001), visual field (p<0.001) and proptosis (p=0.017), respectively. Predictors for PFS were Simpson grade (p=0.048) and number of meningioma tumours (p=0.017). CONCLUSION: In our cohort, all visual outcomes and proptosis improved significantly after surgery. We recommend early surgery after diagnosis, as patients who present with greatly impaired or deteriorated visual function are less likely to have normal postoperative visual outcomes. In addition, we recommend more frequent and tailored follow-up for patients after a Simpson grade II-V resection of the SOM or with multiple meningioma.

MNGI-25. THE CAREGIVER BURDEN IN MENINGIOMA: LONG-TERM RESULTS AND ITS EFFECTS ON CAREGIVER'S HEALTH-RELATED QUALITY OF LIFE, ANXIETY AND DEPRESSION

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BACKGROUND: Various studies in oncological/neurological patients highlight that informal caregivers suffer from a significant disease burden. We aimed to assess the meningioma caregiver burden, and its effects on caregivers health-related quality of life (HRQoL), and levels of anxiety and depression. METHODS: In a multicentre cross-sectional study informal caregivers of intracranial meningioma patients at a median of 10 years after their last anti-tumour therapy were included. Informal caregivers were family members or close friends and completed the caregiver disease burden scale, SF-36 (HRQoL) and the Hospital Anxiety and Depression Scale. Caregiver burden was assessed as an independent determinant for caregivers HRQoL, and levels of anxiety and depression with multivariable analysis correcting for relevant confounders. Participant recruitment is still in progress. RESULTS: 110 informal caregivers were included (mean age: 64.5, female: 37.2%). Informal caregivers reported any caregiver burden in 35.2% of cases, and clinically relevant burden in 15.7%. More specifically, 20.4% of caregivers suffered from stress, 11.2% from social isolation, 13.0% from feelings of disappointment, 21.0% from emotional problems, and 12.0% from environmental factors complicating the care for the patient. The total caregiver burden score was significantly associated with decreased HRQoL on 6/8 scales and 2/2 component scores: physical function (=-6.53, p=0.071), role limitation due to physical problems (=-13.62, p=0.041), bodily pain (=-13.11, p=0.014), social function (=-11.87, p=0.001), mental health (=-14.32, p<0.001), vitality (=-14.24, p=0.001), physical component scale (=-4.40, p=0.022), and mental component scale (=-6.09, p<0.001). In addition, the total caregiver burden score was independently associated with higher anxiety (=3.11, p<0.001) and depression levels (=4.11, p<0.001). CONCLUSION: Caregiver burden in informal caregivers of meningioma patients is considerable, negatively affected their HRQoL, and levels of anxiety and depression. Paying more attention to meningioma patients caregivers seems therefore warranted, and effective support should be implemented if required.

MNGI-26. THE DISEASE BURDEN OF MENINGIOMA PATIENTS: LONG-TERM RESULTS ON WORK PRODUCTIVITY AND HEALTHCARE CONSUMPTION

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BACKGROUND: Meningioma patients suffer from short- and long-term neurological sequelae and impaired health-related quality of life (HRQoL). However, it is unknown how these impairments affect patients work productivity and healthcare consumption on the long-term. METHODS: In a multicentre cross-sectional study intracranial meningioma patients of working age (18–67 years) at a median of 10.0 years after anti-tumour therapy were included. Patients completed a validated questionnaire on work productivity (SF-HLQ), and a study-specific questionnaire on healthcare consumption. One-sample t-test was used to compare meningioma patients with normative data of the Dutch population. Generalised linear models were used to compare meningioma patients with a control population, corrected for: age, sex, educational level and comorbidity. Patient recruitment and data collection is still in progress. RESULTS: 106 meningioma patients were included (mean age: 57.7 years, WHO grade I: 93.5%, surgery: 94.2%, radiotherapy: 17.5%). Meningioma patients had a paid job in 48.1% of cases, compared with 71.8% of the Dutch population of working age (p<0.001). More patients reported obstacles at work (49.1%) than controls (n=72, 20.9%, p=0.003). These problems at work included concentration problems (sometimes: 40.0%, often: 28.0%, always: 8.0%), slower work pace (sometimes: 36.0%, often: 36.0%, always: 12.0%), feeling of isolation (sometimes: 12.0%, often: 12.0%, always: 0.0%), delaying work (sometimes: 52.0%, often: 16.0%, always: 4.0%), and the need for someone to take over their work (sometimes: 33.3%, often: 4.2%, always: 4.2%). Furthermore, on the long-term, specialised healthcare consumption (i.e. outpatient clinic visits) was significantly higher in meningioma patients (70.4%) compared with controls (49.5%, p=0.003). CONCLUSION: Meningioma patients have less often a paid job when compared to the average Dutch population. Moreover, those patients having paid work report more obstacles, particularly concentration problems and a slower work pace. Therefore, employment issues in meningioma should receive more attention, even up to 10 years after intervention.

MNGI-27. THE LONG-TERM DISEASE BURDEN OF MENINGIOMA PATIENTS: RESULTS ON HEALTH-RELATED QUALITY OF LIFE, COGNITIVE FUNCTION, ANXIETY AND DEPRESSION

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BACKGROUND: Previous studies reported that meningioma patients have impaired health-related quality of life (HRQoL) and cognitive function up to 5 years after intervention. We aimed to assess the long-term disease burden of meningioma patients. METHODS: In this multicenter cross-sectional study, intracranial meningioma patients at a median of 9.9 years after anti-tumor therapy were included. HRQoL was meas-

ured with the SF-36 and EORTC QLQ-BN20, anxiety and depression with the Hospital Anxiety and Depression Scale (11/21 points is indicative for probable anxiety or depression), and objective cognitive functioning on six domains (z-score< -1.5 is defined as a clinically relevant deficit). Generalized linear models were used to compare meningioma patients with healthy controls, corrected for age, sex, educational level and comorbidities, and one-sample t-tests to compare meningioma patients with data of newly diagnosed glioblastoma. Patient recruitment and data collection is still in progress. RESULTS: 164 meningioma patients were included (mean age:63.9 years, WHO grade I:91.7%, surgery:89.2%, radiotherapy:14.6%). Compared with 110 controls, meningioma patients had worse HRQoL scores on 4/10 SF-36 scales/component scores: role limitations due to physical problems and emotional problems, social functioning, and the mental component score (range mean difference: 4.4–13.8, all p<0.05). Meningioma patients scored similar to glioblastoma on 5/11 EORTC QLQ-BN20 items/scale (visual deficits, headache, seizure, drowsiness, and hair loss; range difference: 1.6–3.7, all p>0.05), and better on all other items/scales. More patients suffered from probable anxiety (14.3%) and depression (7.5%) than controls (anxiety: 3.7%, p=0.015; depression: 7.5%, p=0.011). Clinically relevant cognitive deficits were found in executive function (10.9%), verbal memory (19.4%), working memory (13.4%), attention (10.3%), information processing (25.0%) and psychomotor speed (14.4%). CONCLUSION: Almost 10 years after the last anti-tumor treatment, meningioma disease burden is still significant. A considerable number of patients have impaired HRQoL, suffer from cognitive deficits and report high levels of anxiety and depression.

MNGI-28. CORRELATION OF METHYLATION CLASS AND GENETIC ALTERATIONS WITH PROGRESSION FREE SURVIVAL IN MENINGIOMA

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BACKGROUND: Meningioma is a heterogenous disease and precise molecular characterization may support clinical decision-making. Several recurring gene mutations as well as prognostic relevant DNA-methylation classes were recently identified in meningioma. We aimed to validate the recent findings in an independent cohort. METHODS: Formalin fixed and paraffin emended samples of 127 meningioma patients (Grade I 40.9%; Grade II: 37.8%; Grade III: 21.3%) were retrieved from the Neuro-Biobank, Institute of Neurology, Medical University of Vienna. Methylation classes (MC) were analyzed using 850k EPIC (Illumina, San Diego, CA, USA) according to the Heidelberg Meningioma Classifier. Panel sequencing for genes reported to impact meningioma, namely NF2, TRAF7, KLF4, ARID, SMO, AKT, TERT, PIK3CA and SUFU was performed as previously outlined. The TRAKLS mutation type was characterized by presence of TRAF7, AKT1, KLF4 or/and SMO mutation. Survival data including progression free survival was retrieved from chart review. RESULTS: Meningioma relevant mutations were evident in 96/127 (75.5%) specimens. NF2 (42/127; 33.1%), TRAF7 (40/127; 31.5%), KLF4 (27/127; 21.3%) and ARID (25/127; 19.7%) mutation were the most frequently observed ones. Two or more mutations were observed in 51/127 (40.2%) specimens. MC correlated with presence of target mutations as well as clinical characteristics (p<0.05; Chi Square test). TRAF7, KLF4 and TERT mutations as well as TRAKLS mutation type associated with progression free survival in univariate analysis (<0.05; log rank test), however in multivariable analysis only MC (HR 1.9; 95% CI 1.3–2.8; p=0.001; cox regression model) and presence of TERT mutation HR 24.9; 95% CI 3.9–159.8; p=0.001; cox regression model) remained statically significant. CONCLUSIONS: Molecular profiling including methylation class and genetic aberrations and may facilitate more precise prognostic assessment and identification of potential targets for targeted therapy in meningioma patients.

MNGI-29. PROGNOSTIC VALUE OF c-erbB2/HER2 EXPRESSION IN HUMAN MENINGIOMAS

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Immunohistochemical studies on the clinical significance of c-erbB2/HER2 expression in human meningiomas have shown diverging results. In the present study, 186 human meningiomas underwent immunohistochemical analyses, 129 benign WHO grade I, 56 atypical WHO grade II, and one anaplastic WHO grade III. Antibodies against internal and external domains, as well as against the phosphorylated/activated receptor, were used. All cases were immunoreactive for the antibody directed against the internal domain, whereas approx. 50 % and 10 % were immunoreactive with the antibodies against the extracellular domain and activated receptor, respectively. Normal meninges were immunonegative. In conclusion, c-erbB2/HER2 is generally overexpressed in human meningiomas, however, only the phosphorylated/activated receptor was significantly associated with increased risk of recurrence or overall survival.

MNGI-30. RADIOLOGIC FEATURES ARE PROGNOSTIC FOR CLINICAL OUTCOMES OF CHORDOID MENINGIOMA

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OBJECTIVES: Chordoid meningiomas are a rare histologic variant that follow an aggressive clinical course. Here, we examine histopathologic and radiologic features of chordoid meningiomas to identify risk factors for recurrence. METHODS: Retrospective chart reviews were performed on 11 patients with chordoid meningioma and 15 patients with meningioma with focal chordoid features (<50% chordoid histology) who were treated at a single institution from 2000 to 2015. The median imaging follow-up was 45 months. A blinded radiologic review was performed on chordoid and 224 non-chordoid meningiomas from the same era. RESULTS: Beyond chordoid histology itself, chordoid meningiomas typically lacked high grade histologic features. In contrast, focal chordoid features were found in meningiomas of all grades, and frequently co-occurred with aggressive histologic features (60%). Recursive partitioning analysis delineated chordoid meningiomas from non-chordoid meningiomas based on high apparent diffusion coefficient (ADC, 73% vs 18%, P=0.0007), hyperintensity on T2-weighted magnetic resonance imaging (82% vs 42%, P=0.04), absence of a cerebrospinal fluid cleft sign (45% vs 77%, P=0.02), and indistinct tumor margins (27% vs 8%, P=0.03). Multivariate analysis identified low ADC (RR 29.1, 95% CI 3.5–462.8, P=0.001), peritumoral edema (RR 8.4, 95% CI 1.2–115.0, P=0.03) and skull base location (RR 5.1, 95% CI 1.2–27.1, P=0.03) as prognostic for local recurrence among meningiomas with chordoid histology. The 5-year local freedom from recurrence and disease specific survival among meningiomas with chordoid histology were 52% and 77%, respectively. CONCLUSION: Meningiomas with chordoid histology have radiologic features that delineate them from non-chordoid meningiomas, and low ADC, peritumoral edema and skull base are prognostic for an elevated risk of recurrence.

MNGI-31. pFOXM1 PROTEIN EXPRESSION IN WHO GRADE III MENINGIOMAS CORRELATES WITH CDK4/6 GENOTYPE AND TRANSFORMATION HISTORY

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BACKGROUND: World Health Organization (WHO) Grade III meningiomas are associated with poor clinical outcomes due to high rates of recurrence and limited adjuvant treatment options. The Forkhead Box M1 (FOXM1) transcription factor is a phosphorylation target of CDK4/6 that protects cancer cells from senescence and is upregulated in Grade III meningiomas. We sought to investigate FOXM1 and phosphorylated FOXM1 (pFOXM1) expression in WHO Grade III meningiomas in relation to proliferative index, CDK4/6 cytogenetic profile, and clinical history. METHODS: We examined 38 tissue specimens from 27 patients with Grade III meningiomas. CDK4, CDK6, and CDKN2A cytogenetic profiles were studied using fluorescence in situ hybridization. FOXM1 and pFOXM1 were identified with immunohistochemistry and quantified using imaging analysis software. RESULTS: pFOXM1 nuclear staining (positive nuclei/mm2) correlated with tumor proliferative index in Grade III meningiomas (mitotic rate > 20, 115.5 vs. mitotic rate < 20, 34.7, P=0.02). Cytogenetic loss of CDK4/6 was associated with significantly decreased pFOXM1 staining (30.8 vs. 111.1, P=0.017). No significant differences in FOXM1 or pFOXM1 staining were observed with gain/amplification of CDK4/6 or with loss of CDKN2A. Grade I/II meningiomas that transformed to Grade III were accompanied by an increase in pFOXM1 staining compared to their de novo counterparts (138.3 vs. 45.9, P < 0.05). CONCLUSIONS: pFOXM1 expression correlated with tumor proliferative rate, CDK4/6 cytogenetic status, and prior Grade I/II meningiomas, suggesting a role of FOXM1 signaling in the transformation of lower-grade meningiomas to Grade III lesions. These findings support the potential role for selective CDK4/6 inhibitors as an adjuvant therapy for Grade III meningiomas.

MNGI-32. LONG-TERM COGNITIVE OUTCOME OF MENINGIOMA AND THE EFFECTS OF TREATMENT (COMET) STUDY RESULTS

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OBJECTIVES: Meningioma is the most common extra-axial tumour, however, little is known about the effect of the disease and its treatment on the brain, and patients functional and cognitive outcomes. We hypothesised that peri-operative factors can effect patients cognitive outcome. DESIGN: We aimed to identify clinical and radiological features associated with cognitive outcome in patients who had undergone resections of a supratentorial meningioma. Subjects: 28 active participants with both complete cognitive and radiological outcomes were identified in our database of focal brain injuries. The cognitive tests performed were the National Adult Reading Test (NART) and Cattell Culture Fair Test (CCFT). Methods: NART and CCFT scores were converted into intelligence quotient (IQ) scores, for use as markers of cognitive outcome. Independent t-tests and ANOVA statistics were utilised to identify features associated with lower IQ scores. RESULTS: There was a negative correlation between age and CCFT IQ scores (r=-0.632, P=0.007). Factors predisposing patients to poorer cognitive outcomes included: recurrent tumour (P<0.05); surgical complications, e.g. infection (P<0.10); additional neurosurgical intervention for complications, e.g. haematoma (P<0.05); and haemosiderin deposition on post-operative radiology (P<0.05). CONCLUSIONS: Peri-operative features can predict patients cognitive outcome. Cognitive dysfunction after meningioma surgery may be more common than currently appreciated.

MNGI-33. RESULTS OF TRANSCRANIAL RESECTION OF OLFACTORY GROOVE MENINGIOMAS IN RELATION TO IMAGING-BASED CASE SELECTION CRITERIA FOR THE ENDOSCOPIC APPROACH

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BACKGROUND: Endoscopic endonasal surgery (EES) is increasingly used for Olfactory Groove Meningiomas (OGMs). The role of EES for large (4cm) or complex OGMs is debated. Specific imaging features have been reported to affect the degree of gross total resection (GTR) and complications following EES for OGMs. The influence of these factors on transcranial resection (TCR) is unknown. OBJECTIVE: We examined the impact of specific imaging features on outcome following TCR to provide a standard for large and endoscopically less favorable OGMs against which endoscopic outcomes can be compared. METHODS: Retrospective study of patients undergoing TCR for OGMs 2002–2016. Results: 50 patients (mean age 62.1 years, mean maximum tumor diameter 5.04 cm and average tumor volume of 48.8 cm3) were studied. Simpson grade 1 and 2 resections were achieved in 80% and 12%, respectively. A favorable functional outcome [modified Rankin Scale (mRS) 0–2] was attained in 86%. The degree of resection, mRS, mortality (4%), recurrence (6%), infection (8%) and CSF leak requiring intervention (12%) was not associated with tumor calcification, absence of cortical cuff, T2 hyperintensity, tumor configuration, tumor extension beyond midpoint of superior orbital roof or extension to posterior wall of frontal sinus. There was no difference in resection rates but a trend towards greater complications between three arbitrarily divided groups of large meningiomas of increasing complexity based on extensive extension or vascular adherence. CONCLUSION: Favorable outcomes can be achieved with TCR for large and complex OGMs. Factors which may preclude endoscopic resection do not negatively affect outcome following TCR.

MNGI-34. THE ROLE OF ALANINE AS A POTENTIAL METABOLIC MARKER IN DETECTING ATYPICAL MENINGIOMAS

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Meningiomas are tumors arising from meninges, the membranes surrounding the brain and the spinal cord. Majority of meningiomas (especially the grade-1) are benign and grow slowly. However, atypical meningiomas (grade-2) exhibit increased cellular proliferation, and grow at much faster rate than benign meningiomas. Moreover, atypical meningiomas have higher probability to recur compared to benign meningiomas. The goal of this study is to identify potential metabolic markers that can be used to distinguish meningioma tumors of grade-1 and atypical grade-2. We have collected tumor tissues from fifteen meningioma patients (grade-1 = 10 and grade-2 = 5) during surgical removal of the tumor mass at the Houston Methodist Hospital. We have employed ex vivo 1H magnetic resonance spectroscopy of tumor tissue extracts to determine whether there are any metabolic differences between grade-1 and grade-2 meningiomas. We have detected the following aqueous metabolites in the tumors that we have studied: leucine, isoleucine, valine, lactate, alanine, acetate, glutamate, succinate, glutamine, aspartate, creatine. phosphocreatine, phosphocholine/glycerophosphocholine, glycine, phosphoethanolamine, myoinositol, and glucose. Quantitative analysis of these metabolites revealed for the first time that alanine and glutamine were highly elevated in grade-2 meningiomas compared to grade-1 tumors. The levels of alanine and glutamine in grade-1 are 2.93 ± 1.20 and 4.81 ± 2.37 µmol/g, while in grade-2 tumors, the levels are 4.95 ± 2.47 and 7.13 ± 4.23 µmol/g respectively. Elevated levels of alanine has been detected previously in grade -1 meningioma patients. However, there has been no study to date that compares various metabolite levels between grade-1 and grade-2 (atypical) meningiomas. Glutamine-derived glutamate is known as the key precursor in alanine biosynthesis. We examine using a larger patient cohort whether there is any correlation between the levels of alanine and glutamine in meningiomas of these two grades.

MNGI-35. SIMPSON GRADING REVISITED: SURGEONS ESTIMATION OF MENINGIOMA REMOVAL VS. POSTOPERATIVE ^{68}GA-DOTATATE PET/CT

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OBJECTIVE: The surgeons intraoperative estimation of meningioma removal (Simpson-Score, SimS) has been accepted as prognostic factor for tumor recurrence with SimS grade I and II being regarded as complete tumor removal. 68Ga-DOTATATE PET/CT has been shown to detect meningioma tissue even more sensitive and specific than MRI. We evaluate the Simpson grading within the framework of modern imaging techniques in both, a retrospective and a prospective data set. METHODS: 37 adult patients with primary or recurrent WHO°I meningioma and surgical resection were retrospectively investigated. The prospective data set comprised 56 patients (59 tumors). Inclusion criteria were documented SimS, postoperative MRI and 68Ga-DOTATATE PET/CT scan. The PET parameters SUVmax, SUVmean and threshold-based biological tumor volume (BTV; SUV>2.3) were assessed by two independent experienced raters. RESULTS: Retrospective cohort: 4 SimS I, 4 SimS II, 4 SimS III and 25 SimS IV resections. 5/8 cases with SimS I and II presented with high tracer uptake thus indicating residual tumor (p=0,0024). Of SimS III and IV cases, all showed pathological 68Ga-DOTATATE-uptake, as expected. In the prospective data set, there were 25/59 resections classified as SimS I, 15/59 as SimS II, 5/59 SimS III and 14/59 as SimS IV. In 15/40 SimS I or II resections, PET displayed tracer uptake indicating unexpected tumor remnants (37% false negative grading in Sims I and II). CONCLUSION: 68Ga-DOTATATE-PET/CT seems to provide more specific information regarding postoperative remaining meningioma tissue compared to the surgeons estimated SimS, especially in cases judged as Simpson I and II resections. This should be taken into account for follow-up management, subsequent therapies as well as in clinical studies.

MNGI-36. BRAIN INVASION IN MENINGIOMAS PREVIOUSLY CLASSIFIED AS WHO GRADE I HAS LIMITED IMPACT ON OUTCOME

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INTRODUCTION: The revision of the WHO classification in 2016 introduced brain invasion as a per se sufficient condition for meningiomas to be classified as grade II. We analyzed whether meningiomas previously graded as WHO grade I according to the 2007 version differ in prognosis solely according to the feature of brain invasion. METHODS: From 1/2009 - 03/2016 all consecutive patients with surgery of a meningioma WHO grade I (2007 grading system) were included. Reference point of the study was date of surgery. Brain invasion was re-evaluated in all cases by a neuropathologist being blinded for the clinical course. Date of last follow up was 11/2017. Study endpoint was the date of tumor progression. Prognostic factors were obtained from multivariate proportional hazards

models. RESULTS: 949 adult patients were included. Median follow-up was 43 months (range: 3–78 months). Gross total resection (GTR) was achieved in 719 (75.8%) patients, adjuvant radiotherapy was applied in 91 (9.9%) patients. Brain invasion was diagnosed by histology in 24 (2.5%) patients. Patients with/without brain invasion did not differ in terms of age and extent of resection. Patients with histologically proven brain invasion received more often adjuvant radiotherapy (p=0.02). 88 patients experienced tumor recurrence, overall survival was not reached in the majority of patients. The overall median PFS for patients with (without) brain invasion was 22 (23) months (p=0.48). In Subtotal resection and history of another malignancy were associated with shorter PFS in univariate and multivariate models (p<0.001), but neither brain invasion nor radiotherapy. CONCLUSION: Among meningiomas classified as grade I according to the WHO 2007 grading system, histological proven brain invasion is rare and was not associated with worse prognosis in contrast to extent of resection and history of another malignancy. However, whether postoperative radiotherapy might have been beneficial for these patients has to be analysed prospectively.

MNGI-37. DMD GENOMIC DELETIONS CHARACTERIZE A SUBSET OF PROGRESSIVE/HIGHER-GRADE MENINGIOMAS WITH POOR OUTCOME

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Progressive meningiomas that have failed surgery and radiation have poor a prognosis and no standard therapy. While meningiomas are more common in females overall, progressive meningiomas are enriched in males. We performed a comprehensive molecular characterization of 169 meningiomas from progressive/high-grade tumors, including matched primary and recurrent samples. We detected frequent alterations in genes residing on the X-chromosome, with somatic intragenic deletions of the dystrophin-encoding and muscular dystrophy-associated DMD gene as the most common alteration (n= 5, 20.8%), along with alterations of other known X-linked cancer-related genes KDM6A (n= 2, 8.3%), DDX3X, RBM10 and STAG2 (n= 1, 4.1% each). DMD inactivation (by genomic deletion or loss of protein expression) was detected in 17/53 progressive meningioma patients (32%). Importantly, patients with tumors harboring DMD inactivation had a shorter overall survival (OS) than their wild-type counterparts [5.1 years (95% CI 1.3–9.0) vs. median not reached (95% CI 2.9–not reached), p=0.006)]. We also assessed for TERT alterations, which have a known poor prognostic association, and found seven patients with TERT promoter mutations and three with TERT rearrangements in this cohort (n= 10, 18.8%), including a recurrent novel RETREG1-TERT rearrangement that was present in two patients. In a multivariate model, DMD inactivation (p=0.033, HR=2.6, 95% CI 1.0–6.6) and TERT alterations (p= 0.005, HR= 3.8, 95% CI 1.5- 9.9) were mutually independent in predicting unfavorable outcomes. Thus, alterations of the mesodermal gene DMD identify a subset of progressive/high-grade meningiomas with worse outcomes.

MNGI-38. LONG-TERM PROGNOSIS OF ATYPICAL AND MALIGNANT MENINGIOMA: OUTCOME AND PROGNOSTIC FACTORS

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AIMS: Twenty one atypical and fifteen malignant meningiomas were analyzed for long time to understand the long-term outcome and associated prognostic factors retrospectively.

METHODS: Thirty three (92%) meningiomas were macroscopically complete-resected in Simpson Grade I resection and three (8%) meningiomas were resected in Simpson Grade II. Ninety-one percent of all patients received whole brain radiotherapy(WBRT) with nean dose of 52 Gy. We analyze the long-term survival, recurrence-free survival and prognostic significance of the grade of surgical resection and the difference between two groups. RESULTS: Recurrence free survival and median time to recurrence were significantly longer in atypical than malignant meningioma. But, the benefit of adjuvant radiotherapy was not effective significantly. Also the grade of surgical resection (Simpson Grade I vs II-III) were significantly related to prognostic survival. CONCLUSIONS: Pathological grade and the grade of surgical resection can be definite prognostic factors. However, multicenter prospective studies are necessary to clarify the management and the correct timing of radiotherapy in such a rare disease.

MNGI-40. MENINGIOMA RESECTION SURGERY IN ELDERLY PATIENTS: AN ANALYSIS OF A NATIONAL DATABASE

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OBJECTIVE: Meningiomas are often benign and mostly asymptomatic, and the treatment approaches may include open surgical resection, radiosurgery, and/or watchful waiting. Reported morbidity and mortality rates for elderly patients undergoing meningioma resection vary widely. We sought to investigate mortality rates for elderly patients undergoing craniotomy for meningioma resection using the Nationwide Inpatient Sample. METHODS: The Nationwide Inpatient Sample (NIS) datasets from 2003 to 2013 were used to identify patient admissions for meningioma resection based on the ICD-9-CM code 01.51. Age categories were defined as 70 years of age. Primary outcomes were in-hospital mortality, poor outcomes (defined as death or discharge to a facility other than home), cost and length of hospitalization. RESULTS: A total of 24,953 patients were identified who underwent meningioma resection during 2003–2013 of which 20.4% were elderly (>70 years). Each of the primary outcomes was heavily influenced by the advancing age. In-patient mortality was higher in the elderly as compared to the younger patients (3.5% vs 1%), as was the rate of a poor outcome (64.8% vs 28.1%). Elderly patients also had a higher cost ($104425 vs $96012) and length of hospitalization (8.9 vs 6.8 days). CONCLUSION: In our study, age > 70 was strongly associated with adverse outcomes after meningioma resection. This increased risk should be taken into account when considering surgical intervention in this subgroup; closer perioperative monitoring may be warranted.

MNGI-41. LARGE VOLUME RE-IRRADIATION FOR RECURRENT MENINGIOMA WITH PULSED REDUCED DOSE RATE RADIOTHERAPY

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BACKGROUND: Meningiomas comprise up to 30% of primary brain tumors. The majority of meningioma patients enjoy high rates of control after conventional therapies. However, patients with recurrent disease previously treated with radiotherapy have few options for salvage treatment, and systemic interventions have proven largely ineffective. The aim of this study was to determine whether PRDR radiotherapy was well tolerated and effective in patients with recurrent meningioma. METHODS: We retrospectively identified 8 patients with recurrent intracranial meningioma treated with PRDR from April 2013 to August of 2017 at a single institution. All patients had radiographic and/or pathologic evidence of progression prior to treatment and had previously completed conventional radiotherapy. Relevant patient, tumor, and treatment characteristics were abstracted from the electronic medical record. Acute and late toxicities were graded based on CTCAE 4.0. RESULTS: Of 8 patients, 6 had histologically confirmed atypical meningiomas upon recurrence. All patients were re-treated with IMRT at an apparent dose rate of 0.0667 Gy/min. Median time between radiation courses was 7.7 years. Median PRDR dose was 54 Gy in 27 fractions to a median volume of 261.6 cm^3. Two patients (25%) had in field failure with a median follow up of 23.3 months. PFS at 6 months was 100%. All but one (87.5%) patient was still alive at last follow up. No patient experienced grade ≥2 acute or late toxicities. CONCLUSION: PRDR re-irradiation is a viable option for patients with recurrent meningioma previously treated with radiotherapy. A phase II trial to assess this prospectively is in development.

MOLECULAR PATHOLOGY AND CLASSIFICATION - ADULT AND PEDIATRIC

PATH-01. DEVELOPMENT OF A SENSITIVE MULTIPLEX ASSAY FOR DETECTION OF MUTATIONS IN IDH1 AND IDH2

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Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are the most frequently mutated metabolic genes in human cancer. They encode cytosolic and mitochondrial enzymes that catalyze the conversion of isocitrate to a-ketoglutarate (αKG), a key component in metabolic and cellular pathways including the Krebs cycle. All located within exon 4, IDH1 and IDH2 mutations are found in multiple types of human cancer including, but not limited to, acute myeloid leukemia and gliomas. IDH mutations occur in the vast majority of WHO grade II/III gliomas and secondary GBMs. Here we describe a sensitive and robust single base extension assay to detect mutations affecting amino acids 100, 105, and 132 of IDH1, and amino acids 140 and 172 of IDH2 in human clinical specimens. Accuracy studies using FFPE, blood, bone marrow, and synthetic controls showed 100% concordance in mutant identification, confirmed using orthogonal methods. Repeatability (intra-assay precision) and reproducibility (inter-assay precision) were 100%. The assay can detect reliably the presence of 5% mutation in a wild-type background with input as low as 0.25 ng DNA (FFPE). Glioma FFPE stored at 15–30°C were found to be stable for 90 days. The IDH1/IDH2 assay has been offered as a clinical test based on its performance characteristics. In a set of 289 clinical specimens including glioma and AML, results were obtained in >98%. Consistent with other published findings, the majority of mutations in glioma affected R132 of IDH1, with other mutations less frequently identified. This IDH assay has high sensitivity, can reliably detect mutations in FFPE samples, and can be implemented as part of routine clinical practice.

PATH-02. ASSOCIATION OF IDH1 MUTATION WITH HISTOLOGICAL TYPE IN INDONESIAN GLIOMA

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Glioma is the most common primary central nervous system (CNS) tumor in adults. One of molecular biomarkers of significant interest for glioma is isocitrate dehydrogenase (*IDH*) mutation. IDH1 C.395G>A (R132H) mutation are reported to occur in 55–80% of grade II and III oligodendroglioma and astrocytomas. IDH mutations have an important role in many aspects of glioma, including glioma genesis, patients prognosis, and development of therapeutic strategies. However, information on IDH mutations in gliomas is not yet available in Indonesian population. Seventy-four glioma patients in a reference hospital in Yogyakarta, Indonesia who underwent surgery were recruited. Glioma tissues in the form of paraffin tissue blocks or fresh samples were sliced for hematoxylin eosin staining and immunohistochemical examination. Genomic DNA was extracted from the samples and IDH1 mutation status was analyzed by PCR and nucleotide sequencing. IDH1 C.395G>A (R132H) mutations were detected in 16 (21.6%) of the samples. This mutation rate is lower than the rate previously reported in Asian population. This study also found that 17.5% of astrocytic type of gliomas harboring this mutation compared to 45.45 % in other tumor types. This difference is statistically significant (p=0.037). In conclusion, IDH 1 mutation is found less frequently in Indonesian glioma, and is associated with the histological subtypes.

PATH-03. PROGNOSTIC IMPORTANCE OF TUMOR GRADE IN THE POST-GENOMIC ERA

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INTRODUCTION: In the post-genomic era of glioma biology, an emerging paradigm is that the molecular and genomic features of gliomas may be more important than tumor grade in determining prognosis. Here, we analyze The Cancer Genome Atlas (TCGA) to assess whether isocitrate dehydrogenase mutation (IDHm) and mutated methylguanine methyltransferase (mMGMT) are better prognostic indicators than tumor grade. METHODS: We identified 1,115 astrocytic gliomas of all grades. We assessed survival using univariate and multivariate Cox proportional hazards models. Multivariate models were adjusted for tumor grade, age, Karnofsky's Performance Score (KPS), mMGMT, and IDHm. RESULTS: Pearson's correlation analysis indicated significant pairwise associations between mMGMT and age (r = -0.22), KPS (r = 0.14), and tumor grade (r = -0.32) (all p< 0.05). Similarly, there were significant pairwise associations between IDHm and age (r = -0.60), KPS (r = 0.34), and tumor grade (r = -0.70) (all p< 0.05). Multivariate analysis showed that age, KPS, tumor grade, mMGMT, and IDHm independently contributed to survival prognosis. For mMGMT tumors, the median survival for grade II, III, and IV tumors was 17.10, 12.70, and 8.55 months; and for MGMT unmethylated tumors, was 13.25, 13.75, and 9.30 months, respectively. For IDHm tumors, two distinct survival distributions were observed for each tumor grade. The first distribution with survival < 60 months, exhibited median survival for grades II, III, and IV patients of 14.3, 12.5, and 16.6 months, while patients surviving >= 60 months demonstrated median survivals of 88.10, 75.15, and 91.10 months, respectively. These survival distributions in IDHm survival did not significantly differ. Wild type IDH tumors fell into a single distribution, with median survival of 7.40, 10.40, and 9.70 months for grade II, III, and IV tumors, respectively. Similar survival patterns were observed in the CGGA. CONCLUSION: Survival prognostication requires synthesis of molecular features of tumors with patient characteristics and tumor grade. For IDHm gliomas, however, tumor grade is a pertinent prognostic factor.

PATH-04. MDM2/4 AMPLIFICATION AND RISK OF HYPERPROGRESSION IN HIGH-GRADE GLIOMAS TREATED WITH CHECKPOINT INHIBITORS

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Checkpoint inhibitors are revolutionizing cancer treatment. However, there are recent reports of systemic cancer patients treated with checkpoint inhibitors with associated hyperprogressive disease (HPD), a ≥2-fold increase in tumor growth rate at first post-treatment imaging, and worse outcomes. Some reports have suggested that MDM 2/4 amplification in advanced cancer may correlate with higher risk for HPD. *MDM2/4* amplifications are relatively common and are reported in up to 20% of glioblastomas. We performed a retrospective review to assess the association between *MDM2/4* amplification and HPD in patients with high grade gliomas (HGG) treated with immune checkpoint inhibitors. Of 102 patients with HGG at our institution receiving PD1 inhibitors, 13 patients were identified to have *MDM2/4* amplification. 5 were treated upfront and 8 at recurrence with PD1 inhibitors. 7/8 patients at recurrence received concurrent bevacizumab. MRIs, prior to and following initiation of checkpoint inhibitor therapy, were evaluated for evidence of HPD. 6/13 patients had radiographic progression on the first MRI after initiation of treatment. Of these, 1 met criteria for HPD in the setting of a trial with nivolumab and vorinostat. She later resumed nivolumab and avastin with significant radiographic improvement; however, continued to clinically deteriorate and died 6 weeks later. 4/6 patients had radiographic pseudo-progression with subsequent response or stabilization of disease, 1 with pathologic confirmation after re-resection. 1 continued to progress at a similar rate prior to starting immunotherapy. In this small retrospective cohort, 1 patient with *MDM2/4* amplification had evidence of HPD after starting treatment with Nivolumab. However, concurrent treatment with vorinostat limits the ability to draw conclusions. Preliminarily, it does not seem that these patients need to be excluded from checkpoint inhibitors trials. After final collection of progression and survival data, we will compare the rate of progression of MDM2/4 amplified HGG to non-amplified MDM2/4 tumors.

PATH-05. IMPLEMENTATION OF A TARGETED NEXT-GENERATION SEQUENCING PANEL FOR THE DIAGNOSIS AND PRECISION MEDICINE TREATMENT OF ADULT PATIENTS WITH WHO GRADE IV DIFFUSE GLIOMAS

Yalan Zhang[1], Annette Molinaro[2], Jennifer Clarke[1], Nancy Ann Oberheim Bush[1], Jennie Taylor[1], Nicholas Butowski[1], Susan Chang[1], Manish Aghi[1], Michael McDermott[1], Philip Theodosopoulos[2], Shawn Hervey-Jumper[2], Mitchel Berger[2], Arie Perry[3], Joanna J Phillips[4], Andrew Bollen[1], Tarik Tihan[1], Melike Pekmezci[1] and David Solomon[1]; [1]University of California, San Francisco, San Francisco, CA, USA, [2]Department of Neurological Surgery, University of California, San Francisco, San Francisco, CA, USA, [3]University of California San Francisco, Dept of Pathology, San Francisco, CA, USA, [4]Department of Neurological Surgery, Helen Diller Research Center, University of California San Francisco, San Francisco, CA, USA

BACKGROUND: Analysis of tumors via next-generation sequencing is now routinely used in clinical practice. The UCSF500 NGS panel became available starting in June 2015. In Dec 2017, a glioblastoma precision medicine initiative started at our institution to sequence all newly-diagnosed WHO grade IV diffuse gliomas. We review our experience over a 3-year

period. METHODS: The UCSF500 Cancer Panel assesses approximately 500 cancer-associated genes for mutations, copy number alterations, and structural rearrangements, including fusions. The test can be run on tumor DNA alone or compared with normal DNA, allowing for discrimination of germline variants. Sequencing results are analyzed by a neuropathologist with genomics expertise (D.A.S.). Results from the 165 adult WHO grade IV diffuse glioma cases sequenced to date were analyzed, including 136 glioblastomas, IDH-wildtype; 19 glioblastomas, IDH-mutant; and 10 diffuse midline gliomas, H3 K27M-mutant. RESULTS: Among the 136 IDH-wildtype glioblastomas, the most common alterations were in TERT, EGFR, CDKN2A, PTEN, NF1, TP53, PIK3R1, PDGFRA, CDK4, MDM2, LZTR1, and STAG2. Among the 19 IDH-mutant glioblastomas, the most common additional alterations were in TP53, ATRX, CDKN2A, and PDGFRA. Paired germline sequencing was performed on 71 patients, ten of which were found to harbor a germline mutation associated with increased cancer risk, including the CHEK2, MSH2, and NF1 genes. Somatic hypermutation was present in nine cases, four at initial resection and five at recurrence with a temozolomide-associated mutational signature. Among the four treatment-naïve glioblastomas with hypermutation, two were Lynch syndrome-associated in patients with damaging germline mutations in MSH2, and two were sporadic tumors that harbored somatic mutations in mismatch repair genes. CONCLUSIONS: Genomic profiling in adult glioblastoma patients results in identification of potentially actionable genetic alterations and also previously unknown germline mutations associated with increased cancer risk. A subset of glioblastomas (approximately 5%) harbor somatic hypermutation, indicating potential utility of immune checkpoint inhibition.

PATH-06. QUANTITATIVE ANALYSIS OF MGMT PROMOTER METHYLATION AND ITS PROGNOSTIC VALUE IN GLIOBLASTOMA MULTIFORME (GBM) PATIENTS TREATED WITH ALKYLATING CHEMOTHERAPY- PRELIMINARY REPORT

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OBJECTIVE: To correlate the percentage of MGMT methylation with progression-free survival (PFS) and overall survival (OS) in GBM patients receiving alkylating chemotherapy. BACKGROUND: MGMT promoter methylation is a known favorable factor for patients with GBM to have better response to the treatment with alkylating chemotherapy and better survival outcome. However, in daily practice, patients with very high percentage of MGMT methylation sometimes were observed to have a shorter survival period. This study is to investigate if the strength of the positivity is correlated to the PFS and OS in GBM patients receiving alkylating chemotherapy. METHODS and PATIENTS: Quantitative MGMT methylation measurement was performed. 5% was defined as positive methylation. Seventeen patients with a diagnosis of GBM and methylated MGMT were reviewed retrospectively. Patients were placed into 3 categories based on their MGMT methylation percentages: 5–33%, 34–66%, and 67–100%. The average PFS and OS were calculated for each category. RESULTS: The 6 patients in the 5–33% methylation category had an average PFS of 14.8 months (range 9 to 32) and OS of 27.2 months (range 10 to 42). The 8 patients in the 34–66% methylation category had an average PFS of 23.9 months (range 0 to 73) and OS of 28.1 months (range 1 to 82). The 3 patients in the 67–100% methylation category had an average PFS of 9.6 months (range 2 to 21) and censored OS of 14.7 months (range 2 to 35) as 2 of the 3 are alive. CONCLUSION: Our sample size is too small to provide conclusions. Comparing the first two methylation categories, the extent of MGMT methylation appears positively correlates with PFS (14.8 versus 23.9 months) but not OS of patients (27.2 versus 28.1 months). Data from additional 15 MGMT methylated patients after follow-ups will be added for analysis.

PATH-07. PRONEURAL GLIOMAS ARE ASSOCIATED WITH POOR SURVIVAL AND MORE LIKELY LOCATED IN PROXIMITY TO THE SUB-VENTRICULAR ZONE

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INTRODUCTION: The Cancer Genome Atlas (TCGA) revealed five sub-classes of astrocytic gliomas; four sub-classes defined by RNA expression (proneural, neural, mesenchymal, and classical), and one by isocitrate dehydrogenase mutation (IDHm). These studies demonstrated prognostic differences only with IDH mutation. Using additional patient and clinical characteristics, we determine if there is a difference in survival between the non-IDH mutated molecular sybtypes of GBM, while accounting for patient age, KPS, or tumor grade. METHODS: We identified 1,073 patients with astrocytomas of all grades from TCGA, excluding IDHm tumors to examine the potential association between RNA expression-based subtype classifications without IDHm as a confounder. We assessed survival using univariate and multivariate Cox proportional hazards analyses adjusted for age, KPS, and tumor grade. We also used The Cancer Imaging Archive (TCIA) to examine the relationship between molecular subtype and propensity for neuroanatomic location of glioblastomas (GBM). RESULTS: Univariate analyses indicated improved survival with increasing KPS (HR = 0.961, p< 0.001), and worse survival with increasing age (HR = 1.054, p< 0.001) and increasing grade (HR = 3.319, p = 0.004 for grade 3; HR = 11.432, p< 0.001 for GBM; relative to grade 2). While no survival association was observed with regards to the RNA-based subtype classification in univariate analysis, in a multivariate analysis that included age, KPS, tumor grade, and RNA-based subtype classification, proneural glioblastomas are associated with worse survival (HR = 1.524, p = 0.012) relative to the non-proneural glioblastomas. Additionally, analysis of TCIA demonstrated that proneural glioblastomas were more likely to be located near the sub-ventricular zone (SVZ, p< 0.05). CONCLUSION: Our findings suggest that RNA expression-based subtype classification has prognostic utility, and proneural subtype of astrocytoma is associated with worse survival. This subtype was more likely to be located near the SVZ, suggesting potential mechanistic insights for this survival association.

PATH-08. THE IVY GLIOBLASTOMA PATIENT ATLAS - A NOVEL CLINICAL AND RADIO-GENOMICS RESOURCE FOR EARLY PHASE CLINICAL TRIAL DESIGN AND INTERPRETATION

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Newly diagnosed GBM represents a population of increased focus in early phase clinical trials. However, a key limitation of current genomic databases of GBM, such as TCGA, is that patient populations eligible for inclusion in these databases exhibit inherent biases and exhibit limitations on the quality of clinical and imaging data available for integration with genomics. To address these limitations and to better represent the genomics of patient populations commonly enrolled to early phase clinical trials, we prospectively consented and enrolled GBM patients to the Ivy Foundation Glioblastoma Patient Atlas Project. A total of 1591 patients from 7 participating sites of the Ben and Catherine Ivy Foundation Consortium for Early Phase Clinical Trials were consented to the project and clinical data was entered into a centrally managed clinical trials database. Overall 658 subjects had pre- and post-surgical imaging centrally reviewed and recorded and 387 subjects had sufficient tissue for completion of targeted exome sequencing of approximately 500 cancer causing genes (Oncopanel or Impact). More than 308 subjects had a complete set of genomics, imaging, and clinical data, including TMZ/RT use, KPS, progression, and steroid use. Histopathological features, MGMT, and IDH mutation status were also annotated. Of the subjects with full clinical data, 171 had expired by the time of last analysis of the cohort. Genomic and clinical characteristics unique to the early phase clinical trial population compared to TCGA and other cohorts of GBM were identified and radio-genomic and other advanced population-based analyses were performed. All clinical, genomic and imaging data are being utilized to create an Ivy cBio Portal for sharing of this rich dataset within the neurooncology community.

PATH-09. CLINICAL CHARACTERISTICS OF ADULTS WITH H3 K27M-MUTANT GLIOMAS AT UCSF

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BACKGROUND: Histone H3.3 or H3.1 mutant protein is commonly expressed in pediatric and adult diffuse midline gliomas, including diffuse intrinsic pontine gliomas (DIPGs), and portends a poor prognosis, regardless of histologic features. As such, "Diffuse midline glioma, H3-K27M-mutant" (DMG-H3K27M) was added to the 2016 WHO Classification as a grade IV entity. Knowledge of the clinical experience and natural history of this recently defined tumor in adults is limited. METHODS: We retrospectively reviewed the pathology of adult (age ≥ 18) DMG-H3K27Ms diagnosed at our institution either via H3-K27M mutant-specific immunohistochemistry or via the UCSF500 targeted next-generation sequencing panel that includes the *H3F3A*, *HIST1H3B*, and *HIST1H3C* genes. Treatment, outcome, and imaging characteristics were reviewed. RESULTS: We identified 26 adults with DMG-H3K27M and 2 with non-midline-H3K27M. Tumor locations included thalamus/basal ganglia (15), hypothalamus (2), pineal region (1), cerebellum (3), brainstem (2), spinal cord (2), mesial temporal (1), and non-midline sites (2). MRI imaging for 21/25 evaluable cases demonstrated enhancement. Of the 26 DMG-H3K27M cases, median age was 35 years (22–68 years). Of these, 17 patients had biopsy only. Median OS was 41 months (95%-CI 31-NA). In the 22 DMG-H3K27M patients with available clinical treatment data, 21 received radiation (19 with temozolomide) at initial diagnosis. At progression/recurrence, 11 patients received bevacizumab, 5 were re-treated with temozolomide, 8 received other chemotherapy, and 8 received > 1 course of re-irradiation. CONCLUSION: While still poor overall, clinical outcome in adults with DMG-H3K27M is often better than that of pediatric DIPGs and other IDH-wildtype high-grade gliomas, such as glioblastoma. This may reflect a different cell of origin or other distinct biologic differences. Further investigation of both DMG-H3K27M and non-midline H3K27M mutant tumors in adults is warranted to study the genetic and epigenetic features of these rare tumors, as well as optimal treatment strategies.

PATH-10. COPY NUMBER (CN)/SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MICROARRAY ANALYSIS OF THE EGFR LOCUS IN GLIOSARCOMA

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Epidermal growth factor receptor (EGFR) is overexpressed or mutated in a variety of malignancies, most notably non-small cell lung cancer, colorectal cancer and glioblastoma (GBM). Glioblastoma is an aggressive primary brain tumor and 35–50% of glioblastomas show amplification of the *EGFR* locus (7p11.2). Interestingly, gliosarcoma, a histologic variant of GBM, has a lower frequency of *EGFR* alterations (4–8%). We characterized *EGFR* alterations in gliosarcoma using a DNA copy number/single nucleotide polymorphism cytogenomic microarray using formalin fixed paraffin embedded tissue. A retrospective search for "gliosarcoma" from our database yielded 19 cases on which microarray analysis was performed. Of these cases, 2 showed an amplification at the *EGFR* locus (13%), 5 cases showed a gain of the entire chromosome 7 (26.3%), 3 cases showed gains at loci other than *EGFR* (15.8%) and the remaining 9 cases were negative for chromosome 7 alterations (47.4%). Our preliminary data show that amplification of the *EGFR* locus are infrequent (13%) in gliosarcomas. These preliminary findings demonstrate antithetical results regarding *EGFR* amplifications in conventional glioblastoma compared to gliosarcoma and suggests there may be an alternate driver in gliosarcoma genesis.

PATH-11. TRANSLATING GENOMIC DATA OF GLIOBLASTOMA INTO CLINICAL PRACTICE: A CASE STUDY

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Glioblastoma (GBM), a malignant brain tumour that occur in adults and children, represents a major challenge for treatment. Tumor heterogeneity has been shown to contribute to this problem. The aim of this study was to overcome this issue by exploring an individualized treatment approach by selecting treatment options using whole genome sequencing, drug-screening panel and a network analysis. We present a case of a 51-year old female long-term GBM survivor with an unmethylated MGMT promoter gene who survived more than three years. Whole genome sequencing (WGS) revealed an ultra-mutated genotype in both primary and recurrent tumour samples with 421 substitutions per megabase. In depth analysis of the WGS revealed an average of 30 cancer driver genes were mutated with a 91% similarity in both primary and recurrent tumors. A drug screening panel and network analysis helped identify actionable targets. The drug screening panel included 165 compounds, of these we identified YM155, an experimental survivin inhibitor as a potential treatment. On the other hand, the network analysis revealed over 130 interconnected pathways affected by mutations in the driver genes. Pathways of interest were selected based on an FDR (false discovery rate) of 0.05 or less. These pathways included PTEN/PI3K/AKT pathway, DNA repair pathway, cell cycle pathway and various signaling pathways. EGFR was found to be predominant in 37% of the affected pathways. Hence, an EGFR inhibitor was recommended for treatment. Genome-guided treatment selection to individualize treatment for GBM patients was demonstrated to be possible in clinic. It remains a promising avenue for further translational research, with larger databases and integrated platforms to increase the efficiency of analyzing and interpreting the individual genomic data of GBM.

PATH-12. CHARACTERISTICS OF GIANT CELL MORPHOLOGY IN LONG-TERM SURVIVORS OF GLIOBLASTOMA: CONSIDERATION OF SEX DIFFERENCES

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The hallmark of glioblastoma (GBM) is poor survivorship. However, a small subset (5%) of patients live greater than 5 years (extreme survivors (ES)). The pathological and tumor determinants of ES are unknown. Giant cell (GC) morphology occurs in < 1% of all GBM, is typically IDH1 wildtype, shows a high frequency of p53 mutations, and is reportedly associated with a somewhat better survival than other IDH1-wildtype GBM. However, the clear association between ES and GC GBM has not be established. We aimed to describe the characteristics of ES tumors that presented with GC GBM features and examine sex differences. In our retrospective multi-institutional database, we identified 90 ES patients with GBM. We reviewed neuropathological reports for the diagnosis of GC GBM or pathology description that included the descriptor(s) of GC: monstrocellular or giant cell or bizarre multinucleated and evaluated phenotypical features in order to describe ES with GC GBM. Values are presented as means. Sixteen (17.8%) ES patients were characterized as GC GBM (males (n=9, 56.25%), females (n=7, 43.75%)). Males were significantly younger than females (39.67 vs 56.29, p=0.018). Females presented with significantly smaller tumors than males (F: 9.49 mm, M: 21.14 mm, p=0.0008). Males less often presented with seizures (33.33%) compared to females (42.86%, p=0.7686). Calculations of invasion/proliferation ratios from our mathematical model revealed no statistically significant differences between females and males. Overall survival between males and females was not statistically significant. Across the board, GC GBM occurred most often in frontal lobes (All: 56% (n=9), 66.67% of males (n=6), and 42.86% of females (n=3)). Extreme survivors of GBM exhibit giant cell features at greater than five-fold incidence than the general GBM population. Sex differences warrant significant attention in future explorations of pathological and tumor characteristics in GC GBM.

PATH-13. THE ORIGIN OF HUMAN GLIOBLASTOMA (IDH WILDTYPE) IS NOT THE LOCATION OF THE TUMOR BUT THE SUBVENTRICULAR ZONE

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Two hypotheses about the origin of human glioblastoma (GBM) genesis are known to be dedifferentiation of cancer cells and orthodox differenti-

ation of cancer stem cells. However, little is known about whether the direction of the human GBM genesis begins at the site of the tumor or at the subventricular zone (SVZ) where normal neural stem cells (NSCs) are present. Here, we describe direct molecular genetic evidence from patient brain tissue and genome-edited mouse models that show astrocyte-like NSCs in the SVZ to be the cell of origin that harbors the driver mutations of human GBM. First, we performed deep sequencing of triple-matched tissues, consisting of i) normal SVZ tissue away from the tumor mass, ii) tumor tissue, and iii) normal cortical tissue (or blood), from 28 patients with GBM, isocitrate dehydrogenase-wild type (IDH-wildtype) or other types of brain tumors. In doing so, we found that normal SVZ tissue away from the tumor in 56.3% of GBM IDH-wildtype patients contained low-level GBM driver mutations (down to ~1% of the mutational burden) that were observed at high levels in their matching tumors. Moreover, via single cell sequencing and laser microdissection analysis of patient brain tissue and genome editing of a mouse model, we discovered that astrocyte-like NSCs carrying driver mutations migrate from the SVZ and lead to the development of high-grade malignant gliomas in distant brain regions. Altogether, our results highlight NSCs in human SVZ tissue as the cell of origin that harbors the driver mutations of GBM. In addition, the origin of human glioblastoma is not the location of the tumor but the subventricular zone.

PATH-14. SURGICAL STRATEGY FOR LOWER GRADE GLIOMAS USING INTRAOPERATIVE RAPID MOLECULAR AND PATHOLOGICAL DIAGNOSIS

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INTRODUCTION: In the 2016 WHO classification, genetic information was introduced in the pathological diagnosis of gliomas, and the correlation between molecular subtype and prognosis has been shown. High extent of removal (EOR) provides survival benefit for some subtype of lower grade gliomas (LGGs), but it is often difficult to achieve both high EOR and preservation of brain function. Our institute has been working on intraoperative rapid molecular diagnosis of LGGs using HRM method for IDH mutation and immunostaining of p53/ATRX for 1p/19q codeletion for an intraoperative decesion making. We report the clinical results of patients with LGGs treated in our facility and discuss the significance of the surgical strategy based on intraoperative rapid molecular diagnosis. METHODS: In 366 cases (G2: 219 cases, G3: 147 cases) with newly diagnosed LGGs that could be classified according to 2016 WHO classification in our hospital (2004–2014), the relation between the EOR and prognosis was retrospectively analyzed. Accuracy of intraoperative rapid molecular diagnosis of IDH mutation and 1p/19q codeletion using HRM method and p53 /ATRX immunostaining was evaluated. Result: The 10-year survival rate of oligodendroglioma was 88% in G2 (121 cases), 80% in G3 (59 cases), the 10-year survival rate of DA-IDH mutant was 63% in G2 (66 cases), MST of G3 (46 cases) was 13.6 years, MST of DA - IDH wild-type was G2 (32 cases) 12.6 years, G3 (42 cases) 3.9 years. The EOR strongly correlated with prognosis in DA-IDH wild-type. The results of intraoperative IHC of p53/ATRX were 82.6% consistent with FISH based results of 1p/19q codeletion. The HRM results (55 cases) were 100% consistent with sequence based IDH mutation results. Conclusion: The prognosis and the significance of EOR was significantly different between subtypes in LGGs. Rapid intraoperative molecular diagnosis seems to be useful for determining the removal strategy in LGG surgery.

PATH-15. MICROGLIA/MACROPHAGES ARE THE MAJOR TUMOR-ASSOCIATED IMMUNE CELLS IN PILOCYTIC ASTROCYTOMA

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Pilocytic astrocytoma is the most common glioma in children. Although most pilocytic astrocytomas are surgically curable, a small subset does recur and demonstrates CSF dissemination. *BRAF*-targeted therapies have been applied in recurrent pilocytic astrocytomas. However, a small subset of these tumors lack *BRAF* alterations. More recently, there has been an emerging role of tumor-associated immune cells (including microglia/macrophages) in gliomagenesis with an attempt to find additional targets as standalone or combined adjuvant therapy. In this study, we investigated composition of different tumor-associated immune cells in pilocytic astrocytomas. Tissue microarrays from 55 pilocytic astrocytomas were generated. Immunohistochemistry for CD3, CD20, CD21, CD68, and CD163 was performed to label different tumor-associated immune cells (T-cells, B-cells, dendritic cells, and microglia/macrophages respectively.) Tumor-associated microglia/macrophages were found at moderate to markedly high density in most pilocytic astrocytomas. CD163 and CD68 positive microglia/macrophages comprised >10% of cells in 67% (37/55), and 60% (33/55) cases. These microglia/macrophages presented as either scattered infiltrate and/or perivascular aggregates. In contrast to microglia/macrophages, lymphocytes and dendritic cells were only found at sparse-to-moderate density. In conclusion, our study reveals that microglia/macrophages are the major tumor-associated immune cells in pilocytic astrocytomas, at least in this small cohort, and raises the possibility of extrapolating tumor-associated microglia/macrophages as a potential therapeutic target in pilocytic astrocytomas.

PATH-16. MOLECULAR PATHOLOGY AND CLINICAL CHARACTERISTICS OF MMR DEFICIENCY (MMRd) IN DIFFUSE GLIOMAS

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BACKGROUND: MMR deficiency (MMRd) is found in a subset of gliomas. Emerging evidence indicates that MMRd could serve as predictive biomarker for both response to immunotherapy and resistance to alkylating agents. However, the molecular epidemiology, outcome, and the optimal diagnostic method of MMRd are poorly defined in gliomas. METHODS: A cohort of 350 adult gliomas was characterized as to WHO 2016 diagnosis, IDH1/2 sequencing, MGMT promoter methylation, prior treatment and outcome. Immunohistochemistry of MMR proteins (MSH2, MSH6, MLH1, PMS2) was performed in 260 tumors with available tissue. 100 tumors were analyzed with next generation targeted exome sequencing (NGS, Oncopanel) of 447 cancer genes, including full exon coverage of MMR genes. Comparisons between results from IHC, NGS and microsatellite instability (MSI) testing using pentaplex PCR amplification testing were incorporated to inform best practices. RESULTS: 53/260 tumors showed immunohistochemical (ie MMR protein staining loss) and/or molecular (ie presence of MMR pathogenic mutation with hypermutation) evidence of MMRd. No MSI was found by pentaplex PCR. 38/260 tumors harbored loss of protein expression of one or two MMR proteins: 15 showed loss of MSH6 protein (with/without concomitant MSH2 loss) and 23 had loss of PMS2 (with/without MLH1 loss). Analysis of 201 consecutive recurrent tumors from alkylator pre-treated patients showed significant association between MMRd and the presence of IDH1/2 mutation: MMRd in 20/90 (22.2%) of IDH1/2-mutant tumors vs 2/111 (1.8%) of IDH1/2-wild-type tumors ($p<4.10–6$). In addition, MMRd was found in 6/17 (35%) of patients with de novo gliomas and clinical features indicating possible inherited MMRd. Outcomes and updated results of molecular biomarker analyses will be presented at the conference. CONCLUSIONS: Loss of MMR protein expression by IHC is significantly associated with IDH-mutant relapses and a clinico-histo-molecular presentation suggestive of germline MMRd. NGS and IHC of MMR proteins are highly concordant in gliomas, while MSI testing lacks sensitivity.

PATH-17. INCREASING VALUE OF AUTOPSIES IN PATIENTS WITH BRAIN TUMORS IN THE MOLECULAR ERA

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INTRODUCTION: Many pediatric brain tumors are associated with high morbidity and mortality, which is due to insufficient understanding of tumor biology. Limited tissue allocation for research from small surgical specimens is a key barrier to improved understanding but brain tumor autopsies have

been a valuable resource. This study reviews the brain tumor autopsy practice at our institution, and describes emerging research ultilization patterns beyond the clinical autopsy report. METHODS: Brain tumor autopsies in the interval 2007–2017 were identified, and we analyzed the method of tissue triaging for research and documented its specific uses. RESULTS: Of 1602 deaths at Boston Children's Hospital (636 with autopsies), 96 had a diagnosis of brain tumor (56 consented for autopsy,). Diffuse intrinsic pontine glioma (DIPG) and other high-grade gliomas accounted for the greatest proportion of diagnoses (52% of brain tumor autopsies). The tumors that resulted in the highest number of autopsies were DIPGs (25 deaths, 21 autopsies). Other frequent diagnoses were atypical teratoid rhabdoid tumors (13 deaths, 8 autopsies) and medulloblastomas (12 deaths, 3 autopsies). Fourteen DIPGs (56%) had tissue samples contributed to the DIPG registry consortium. Mapping was performed on 20 DIPG tumors in order to study heterogeneity; ten underwent whole genome sequencing, RNA expression studies and arrayCGH. Cell lines were successfully generated from 2 DIPGs and 1 ATRT (attempted on 12 autopsies) that had a post-mortem interval of less than 8 hours. CONCLUSIONS: Our institutional pediatric brain tumor autopsy experience demonstrates the increasing utility of autopsy-derived tissue for multiple types of research. Our experience demonstrates a wide utilization of brain tumor autopsy material in translational research, and might encourage research consent for brain tumor autopsy and active collection of unfixed autopsy material in the molecular era.

PATH-18. SUBCLASSIFICATION OF LOW-GRADE GLIOMAS CONSIDERING TERT PROMOTER MUTATION AND ATRX LOSS: BEYOND THE 2016 WHO CLASSIFICATION

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BACKGROUND: Grade II glioma is a heterogeneous group of various pathologies. 2016 WHO classification defined subgroups of Grade II gliomas based on isocitrate dehydrogenase (IDH) mutation and 1p/19q codeletion status. However, implications of telomerase reverse transcriptase promoter (TERTp) mutation and alpha-thalassemia/mental retardation syndrome X-linked (*ATRX*) loss are not considered in the classification. METHODS: Patients (n = 191) who underwent surgery and pathologically proven for supratentorial newly diagnosed low-grade glioma (WHO grade II) were included this study. Molecular diagnoses including *IDH1/2* mutation, 1p/19q codeletion, *TERT*p mutation, *ATRX* expression, O^6-methylguanine-DNA methyltransferase (*MGMT*) promoter methylation status was evaluated. The overall survival according to *TERT*p mutation and *ATRX* loss in each 2016 WHO class were compared. RESULTS: There were 34 (17.8%) IDH-wildtype astrocytomas, 81 (42.4%) IDH-mutant astrocytomas, and 76 (39.8%) IDH-mutant and 1p/19q-codeleted oligodendrogliomas. The median overall survival (OS) of each group were 3.9, 10.4, and 18.7 years, respectively. *TERT*p mutation had negative impact for survival in IDH-wildtype astrocytomas (HR = 5.458, 95% confidence interval [CI] 1.771–16.826), while no significant differences were observed regarding *TERT*p mutation in IDH-mutant astrocytomas and oligodendrogliomas. Among IDH-wildtype/TERTp-mutant astrocytomas, *ATRX* loss was significantly correlated with poor outcome (2.1 vs 3.0 years, p=0.033). CONCLUSIONS: Molecular status of *TERT*p mutation and *ATRX* expression can help stratifying IDH-wildtype astrocytomas. IDH-wildtype astrocytomas which harbor TERTp mutation only without *ATRX* loss showed the worst outcome. Further study is needed to verify the role of *TERT*p mutation and *ATRX* in gliomas.

PATH-19. CLINICOPATHOLOGIC FEATURES AND OUTCOMES OF HISTOLOGICALLY CONFIRMED ATYPICAL DIFFUSE INTRINSIC PONTINE GLIOMA

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Diffuse intrinsic pontine glioma (DIPG) is predominantly diagnosed clinically, with biopsy often reserved for cases with atypical imaging features and/or symptoms. Outcome data and molecular profiling of DIPG have been limited almost entirely to tumors with typical features. We examined the clinicopathologic characteristics and outcomes of 28 patients with pontine-centered lesions who underwent diagnostic biopsy at our institution from 2003 to 2018 because of atypical MRI findings. The median age at diagnosis was 5.2 years (range, 0.7–17.1), and the median symptom duration pre-diagnosis was 1 month (range, 0–24). Common atypical features included pontine asymmetry, tumor extension into the medulla and cerebellar peduncles, and exophytism. Four patients (14.3%) developed transient neurologic symptoms; one (3.6%) experienced a new, permanent cranial neuropathy; and no biopsy-related deaths occurred. Sixteen of the 28 tumors (57%) were confirmed histologically as DIPGs (WHO grade II–IV gliomas; "atypical DIPG"). Of these 16 cases, H3 K27M status was assessed through immunohistochemistry in 14 (88%) and nine were immunopositive (64.3%). The remainder ("atypical non-DIPGs") were pilocytic astrocytomas (58.3%), gangliogliomas (8.3%), CNS embryonal tumors, NOS (16.7%), or C19MC-altered embryonal tumors with multilayered rosettes (16.7%). With a median follow-up of 28.6 months (range, 3.0 – 173.2), 2-year overall survival (OS) was 72.4%, 83.3%, and 62.2% for the total cohort, atypical non-DIPGs, and atypical DIPGs, respectively (P= NS for all). However, OS with H3 K27M–mutant atypical DIPG was inferior to that with H3 WT atypical DIPG (P= 0.003). Compared to OS in a contemporary typical DIPG cohort (N= 100), OS was significantly longer for the total atypical cohort, atypical non-DIPGs, and atypical DIPGs (P< 0.001 for all), but not significantly different for H3 K27M–mutant atypical DIPGs. Thus, despite atypical imaging features, most lesions were consistent with DIPG. Biopsy contributed to revised diagnosis and management in a large number of cases. Integrative molecular analyses are ongoing.

PATH-20. ANAPLASTIC ASTROCYTOMA: WHY DOES SURVIVAL DIFFER SO MUCH FOR THE SAME HISTOLOGICAL GRADE?

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BACKGROUND: Increasing knowledge and understanding of the molecular genetics driving both the occurrence and transformation of gliomas has led to a more discrete and objective classification of such tumours. Both phenotypic and genotypic variations now underpin their classification, and thus help to more accurately guide their clinical management. However, WHO Grade III Anaplastic Astroyctoma (AA) remains an unpredictable, heterogeneous entity; displaying a variable prognosis, clinical course and treatment response. AIMS: To examine additional measurable tumour characteristics that may delineate overall survival (OS) more predictably in AA. METHODS: Data was collected on newly diagnosed cases of AA between 2003–2014, followed up for a minimum 3-years. Molecular information was obtained from case records and prospectively performed in cases where missing. Histological slides were manually examined for Ki67 proliferation index, cellularity and number of mitotic figures. Cox-regression and Kaplan-Meier analyses were used to assess OS. RESULTS: In total, 51 cases were included with an OS of 12months (range: 1 – 150months). Cumulative 3-year survival was 29.4%. Median age was 50years (range: 24 – 77years). Across the cohort, age, IDH1m status, oncological therapy and Ki67 were significant independent prognostic indicators on multivariate analysis (p<0.05). Median age in IDH1 wild-type (IDH1wt) tumours was significantly greater than IDH1 mutant cases (p<0.01). In cases demonstrating OS ≥3-years, Ki67 index, number of mitotic figures and percentage areas of 'high cellularity' were significantly reduced i.e. more characteristic of lower-grade/WHO Grade II glioma. Neither age nor Ki67 index had prognostic impact on IDH1wt cases. CONCLUSIONS: IDH1m status remains the most significant prognostic indicator amongst AA but Ki67 index has a significant independent prognostic value in AA. Number of mitotic figures and cellularity also offer valuable prognostic information. Further investigation utilising Ki67 quantification in standard tumour characterisation in histologically confirmed AA may aid in prognostication and inform precision medicine.

PATH-21. ANGIOTENSINOGEN GENE SILENCING PREDICTS BEVACIZUMAB RESPONSE IN RECURRENT GLIOBLASTOMA PATIENTS

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BACKGROUND: Bevacizumab in combination with chemotherapy has shown activity in recurrent glioblastoma patients, with responding patients

having improved survival as well as quality of life. Recently, we found that low gene expression of angiotensinogen (AGT) was predictive for bevacizumab response in recurrent glioblastoma patients. Because promoter methylation of AGT has been associated with AGT gene silencing, we investigated if AGT promoter methylation in tumor tissue predicts response to bevacizumab combination therapy in recurrent glioblastoma patients. METHODS: The study includes 82 recurrent glioblastoma patients treated with bevacizumab combination therapy whom were both RANO response and biomarker evaluable. DNA methylation of 7 CpG sites in the CEBPA binding site (~200 bp from TSS) of the AGT promoter was measured using pyrosequencing. AGT gene expression in tumor tissue was measured by NanoString analysis. For each CpG site, methylation levels were associated with angiotensinogen gene expression using Spearman correlations and to treatment response using Mann-Whitney U test and logistic regression analysis. RESULTS: Preliminary results on 58 of 82 patients analyzed: AGT gene expression was inversely associated with AGT promoter methylation on CpG site 1 (P= 0.049) and borderline significant on CpG site 2 (P= 0.074). Compared to non-responding patients, responders expressed significantly higher methylation levels of CpG site 1 (P= 0.015), 2 (P= 0.013) and 3 (P= 0.045). DNA methylation levels at CpG site 4–7 were not associated with AGT gene expression or response. By univariate analysis, increased methylation of the AGT promoter region were predictive for bevacizumab response on CpG site 1 (2-fold increase: OR= 1.81; 95%CI: 1.02–3.23; P= 0.043) and on CpG site 2 (2-fold increase: OR= 2.08; 95%CI: 1.04–4.17; P= 0.040). CONCLUSION: Increased methylation of the AGT promoter regions is associated with AGT gene silencing and is predictive for bevacizumab response in recurrent glioblastoma patients. Updated results will be presented.

PATH-22. THE DEVELOPMENT OF A NEW ASSAY TO MEASURE 2-HYDROXYGLUTARATE (2-hg) ENANTIOMER LEVELS AND THE UTILITY OF 2-hg AS A BIOMARKER FOR MANAGEMENT OF IDH MUTANT GLIOMAS

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The isocitrate dehydrogenase (IDH) gene is mutated in a large percentage of gliomas and produces the oncometabolite, (R)-2-hydroxgylutarate [(R)-2hg]. However, no correlation between total 2hg levels and clinical parameters, like tumor burden, in glioma patients has been identified. This lack is likely due to the presence of significant levels of the enantiomer, (S)-2-hydroxyglutarate [(S)-2hg]. (S)-2hg is normally produced in the body and during hypoxic conditions. Past studies measuring 2hg via mass spectrometry methods either do not differentiate between (R)-and (S)-2hg, both which are normally detectable in serum, or use LC-MS based assays that lack the sensitivities needed to detect changes in (R)-2hg when an IDH mutant glioma is present. In this study, a novel gas chromatography-mass spectrometry method was built to measure 2hg enantiomers and used to determine if serum levels of (R)-2hg:(S)-2hg, or absolute levels of (R)-2hg, could provide clinical utility as a biomarker in patients with IDH mutant gliomas. The derivatization, fragmentation, and quantitation scheme was optimized for sensitive detection of basal 2hg enantiomers using normal human serum. Serum was then collected from patients with IDHmut and IDHwt gliomas and analyzed to measure levels of 2hg enantiomers before and during the course of treatment. The ratio of (R)-2hg:(S)-2hg was increased in a number of patients with actively growing IDHmut tumors, but not in patients with stable IDHmut tumors or IDHwt tumors. These preliminary results demonstrate the utility of the GC-MS assay for measurement of 2hg enantiomers and their ratio as a biomarker for active disease in patients with IDHmut gliomas.

PATH-23. GERMLINE GNAS MUTATION IN AN 18-MONTH-OLD WITH MEDULLOBLASTOMA

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Medulloblastoma is the most common malignant brain tumor of childhood. It is a molecularly and clinically heterogeneous tumor. There are currently four recognized molecular subtypes of medulloblastoma, one of which is sonic hedgehog (SHH)-activated medulloblastoma. We discuss a case of an 18-month-old male with a desmoplastic SHH-activated TP53-wildtype medulloblastoma, failure to thrive, global developmental delay, and polydactyly found to have a novel de novo heterozygous c.565-568delGACT germline GNAS frameshift mutation on the paternal allele identified through peripheral blood trio clinical exome sequencing. Germline GNAS mutations are known to be associated with several diseases. However, to the authors knowledge, this is only the second report in the literature of a germline GNAS mutation in a patient with medulloblastoma. GNAS is a known tumor suppressor of the SHH pathway. A prior study has shown that low somatic GNAS expression characterizes a subset of patients with aggressive SHH-activated medulloblastoma. The normal function of GNAS encoded Gas is to stimulate adenylyl cyclase activity to produce intracellular cyclic adenosine monophosphate (cAMP), which activates the cAMP-dependent protein kinase A (PKA), and inhibits SHH signaling. Thus, low or loss of GNAS expression leads to aberrant SHH pathway activation. Upregulation of the SHH pathway results in increased granule cell progenitor proliferation and tumor formation. This provides insight into the mechanism by which our patients GNAS mutation may have fueled development of medulloblastoma. Germline GNAS mutation must be considered in patients with SHH-activated medulloblastoma and in particular, in patients with phenotypic similarities to our patient including developmental delay, small size for age, and polydactyly. Advancing our understanding of medulloblastoma development through the study of germline and tumor genomics including better understanding the role of GNAS, holds great promise for enabling improved treatments and patient outcomes.

PATH-24. RECURRENT UNUSUAL PATTERNS IN CLINICAL MOLECULAR PROFILING OF ADULT DIFFUSE GLIOMAS

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Adult diffuse gliomas (ADG) are stratified in clinically relevant groups based on 1p/19q co-deletion status, and *IDH* and *TERT* promoter (*TERT*p) mutational status. Using a clinical NGS assay targeting 50 ADG-associated genes, 419 ADG (at least 18 years; 59 grade II, 131 grade III, 229 grade IV) were profiled within a 15-month period. In a subset of cases, chromosomal microarray (n=132) or 1p/19q FISH (n=19) was performed. There were 89 *IDH*-mutant (33 WHO "Oligodendroglioma, IDH-mutant and 1p/19q co-deleted", 3 "Astrocytoma, IDH-mutant" with TERTp mutation and 53 "Astrocytoma, IDH-mutant") and 330 *IDH* wild-type (254 TERTp-mutant and 76 TERTp wild-type) cases. Mutation pattern analysis was performed using heatmap2 hierarchical clustering. ADG arising within the midline were predominantly "Astrocytoma, IDH-wildtype" (11 *TERT*p-mutant and 10 *TERT*p wild-type cases), with one "Astrocytoma, IDH-mutant" and two "Diffuse midline glioma, H3 K27M-mutant" cases. Unusual recurrent patterns were noteworthy. "Astrocytoma, IDH-mutant" with *TERT*p mutation were 1p/19q-intact lower-grade gliomas that lacked *ATRX* mutations and recurrently showed *TP53* mutations, chromosome 7 gain and *CDKN2A/B* copy-neutral loss-of-heterozygosity. "Astrocytoma, IDH-wildtype" with *TERT*p and *BRAF* mutations had *BRAF* V600E (n=4) and non-V600E (n=3) mutations, and were high-grade tumors with recurrent *PTEN* mutations. A subset of "Astrocytoma, IDH-wildtype" had a single gene mutation (n= 29). Only 9 tumors (2%) were negative for mutations within the 50 interrogated genes. Chromosomal microarray of 6 of these mutation-negative cases revealed genomic abnormalities, including gain/amplification involving *TERT* in addition to copy number changes usually observed in "Astrocytoma, IDH-wildtype". This suggests that such tumors were likely driven mainly by chromosomal instability/copy number abnormalities and that *TERT* copy number changes may represent an alternative telomere maintenance mechanism in ADG. Clinical molecular profiling shows recurrent unusual patterns in ADG and underscores the challenges and the need for large scale initiatives to provide guidance on how to clinically interpret such patterns.

PATH-25. SURVIVAL STRATIFICATION OF IDH MUTANT GLIOMA USING METHYLATION AND mRNA ANALYSIS OF HOX GENES

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Diffuse gliomas are separated based on IDH mutation (mut) status. However, IDH-mut gliomas manifest wide range of clinical outcome that

are not explained by the current genomic classification. We aim to identify clinically and biologically relevant subgroups within IDH-mut low grade gliomas to gain a deeper insight and improve classification. We used 412 IDH-mut gliomas profiled by The Cancer Genome Atlas (TCGA) Network, utilising methylation, mRNA and mutation datasets to identify unique molecular signatures. We found that IDH-mut gliomas further subdivide into 2 groups based on mutational rate. High mutation load predicts poor survival in IDH-mut glioma. Analysis of differentially expressed genes in high versus low-mutational rate showed significant enrichment of HOX genes, 24/40 HOX genes were up regulated in this group. Interestingly, both overexpression and hyper-methylation of specific HOX genes were associated with worse survival. We further show that 7 of these HOX genes (HOXA4, HOXA7, HOXA10, HOXA13, HOXD3, HOXD9, and HOXD10) are the most significant in determining survival. Signed average of 7 Hox genes significantly improved survival and hazard ratio (HR) based on high versus low methylation (HR=4.3, p<0.0001) and high versus low mRNA expression (HR=2.8, p=0.00095). Similarly, effect on survival based on high expression and hyper-methylation of HOX genes was not only observed in IDH-mut 1p/19q-codeleted and non-codeleted groups independently, but also in IDH-wild-type low grade glioma. Multivariate analysis adjusted for confounding factors (grade, age and codeletion status) showed prognostic factors associated with survival in high versus low methylated group (HR=3.2, p=0.0036). Interestingly, only the same direction (high-high and low-low groups) of both mRNA and methylation showed significance and increased HR, which challenges the current understanding of methylation of genes and gene expression. We show that IDH-mut gliomas can further be stratified into clinically relevant categories based on high mRNA expression and hyper-methylation of Hox genes.

PATH-26. NEURO-ONCOLOGY NEXT-GENERATION SEQUENCING 219-GENE PANEL FOR COMPREHENSIVE CLINICAL TESTING

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Molecular parameters were incorporated in the WHO classification of central nervous system (CNS) tumors. Despite advances in understanding the molecular biology of CNS tumors, targetable genomic abnormalities are lacking. To identify diagnostically, prognostically and potential therapeutically relevant abnormalities, we developed a next generation sequencing (NGS) assay for formalin-fixed paraffin-embedded (FFPE) tissue that evaluates 219 genes associated with adult and pediatric CNS tumors. This test consists of a DNA and an RNA subpanel for the detection of sequence alterations and gene rearrangements (known gene fusions and abnormal transcript variants, and novel fusion transcripts that contain any of the interrogated genes as a partner). The assay utilizes an amplicon-based approach with molecular barcode chemistry (to allow traceability of PCR artifacts/duplicates), Illumina sequencing and custom bioinformatics pipelines. Analytical validation included 175 samples. Overall concordance with alternative methods were 99% and 96% and success rates were 97% and 95% for the DNA and RNA subpanels, respectively. Inter and intra-assay reproducibility was 100% for both subpanels. The limit of detection (analytical sensitivity) for nucleic acid input and tumor content were 8.5 ng and 30%, and 10 ng and 10% for the DNA and RNA subpanels, respectively. The analytical specificity was high, with per base DNA sequencing false positive rate <0.4% and absent fusion transcript detection in non-neoplastic samples. We developed a robust 219-gene neuro-oncology NGS assay suitable for clinical testing of FFPE specimens, including small biopsies with low tumor content. This test, combined with chromosomal microarray analysis, detects nearly all single nucleotide variants, fusion rearrangements and copy number changes associated with CNS tumors. These tests are intended to assist in the diagnosis, prognosis and therapeutic management of adult and pediatric patients with CNS tumors, and have the potential to unravel novel genomic abnormalities and expand the understanding of the molecular biology of such tumors.

PATH-27. IDENTIFYING THE GENETIC SIGNATURE OF RESPONSE IN A PHASE II STUDY OF TUMOR TREATING FIELDS IN RECURRENT GLIOBLASTOMA

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Prognosis of relapsed glioblastoma (GBM) is dismal and current treatment fails to provide prolonged survival. Small subsets of patients respond well to some novel therapeutics probably due to the genetic variations of tumors and patients. In the Phase 3 EF-11 recurrent GBM trial, a small subset of patients derived significant benefit from TTFields alone. This proof-of-concept trial [NCT01954576] will study adult patients with relapsed GBM treated with TTFields by genetic analysis of primary and recurrent tumors. Post-hoc correlations will be used between clinical response, mutational analyses and quantitative gene expression to define genomic signatures of response. Whole exome and RNA sequencing will be used to identify genomic signature of responders to TTFields. Fifteen patients with bevacizumab-naïve recurrent GBM and 15 patients with bevacizumab-refractory GBM will be treated with TTFields for 6 and 4 months respectively. Patients will undergo standard brain MRI scans without and with gadolinium contrast and perfusion imaging every 8 weeks. Tissue from the primary tumor at recurrence will be genetically analyzed. Genomic DNA (gDNA) will be extracted from patients tumor and blood samples. Purified gDNA fragments will be used for Illumina sequencing library construction. Certain germ-line variants may contribute to gliomagenesis and be associated with somatic mutations within the tumor and subtypes of GBM more or less sensitive to TTFields. Analyses will be conducted on all patients: bevacizumab-naïve and bevacizumab-refractory GBM separately, and patients with objective radiographic response (complete response + partial response (CR + PR) and stable disease (SD) separately. With 50% bevacizumab-refractory GBM patients, response rate will be significantly higher than the baseline rate of 14%. Using an Exact test with type I error of 0.05 and 80% power, the estimated sample sizes will detect a statistical difference on response rate in the TTFields group compared to historical controls. To-date 4 patients have been enrolled.

PATH-28. THE NATURAL HISTORY OF BRAF V600E-MUTATED GLIOBLASTOMAS IN ADULTS

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BACKGROUND: BRAF V600E-mutations are rare but noteworthy in primary brain tumors given their potential as a targetable mutation and the lack of efficacious therapies for glioblastoma. BRAF V600E mutations may serve as a prognostic marker in pediatric and low-grade gliomas, and are associated with improved survival in young adults with glioblastoma; however, its prognostic significance in adults >35 years is uncertain given the very small number of patients evaluated to date. METHODS: Patients aged >18 with WHO III-IV glioma and a BRAF V600E mutation were identified from the National Institutes of Health, the Johns Hopkins Hospital, and a previous publication (PMID:27503138). Paired control cases were identified at each institution based on age, sex, degree of resection, performance status at diagnosis/first encounter, MGMT and IDH status, and first-line treatment. Log-rank test was used to compare survival curves. RESULTS: The present cohort consisted of 23 patients (6 from each institution and 11 from a published cohort) with median age of 39 (range 20–70 years), 78% female, and 87% with a glioblastoma diagnosis. No tumors had an IDH mutation. 39% of patients were aged >50 years. All but one were treated with radiation and temozolomide at diagnosis (exception went into hospice and died shortly thereafter). The median overall survival was 33.4 ± 8.4 months in all patients. For 13 patients aged 35 or older, median survival was 34.5 ± 12.1 months compared to 18.0 ± 3.0 months in case-matched controls (p=0.03). Two patients were treated with dabrafenib and trametinib; one is still on therapy (26 months), the other progressed after 8 months. CONCLUSIONS: Adults aged >35 with BRAF V600E mutation may have improved survival compared with matched controls, similar to results in young adults. BRAF V600E mutations occur in patients with glioblastoma aged >50 years and testing in this population should be considered as well.

PATH-29. CLINICAL SIGNIFICANCE OF TEMOZOLOMIDE-INDUCED SOMATIC HYPERMUTATION IN INITIALLY LOW-GRADE IDH-MUTANT DIFFUSE GLIOMAS

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INTRODUCTION: Diffuse low-grade gliomas (DLGG) treated with temozolomide (TMZ) can develop somatic hypermutation. We present data on the incidence and prognostic importance of somatic hypermutation in IDH-mutant DLGGs. METHODS: We analyzed 120 patients treated on a phase II clinical trial of TMZ for sub-totally resected DLGGs to estimate the risk of recurrence and transformation after TMZ. To understand the prognostic significance of somatic hypermutation, we determined hypermutation status by exome or targeted sequencing on tumors from 81 patients with recurrent IDH-mutant DLGGs. 63/81 patients received TMZ before recurrence, including 28 patients treated on-trial. RESULTS: With median follow-up of 8.7 years, 89 patients from the phase II trial progressed, 60 underwent 1 re-operation, and 36 had histologically confirmed transformation. The 8-year freedom from transformation was 48.2% and 59.9% for IDH-mutant astrocytomas and oligodendrogliomas, respectively; risk of transformation increased with pre-TMZ tumor volume (HR 2.5 per 100cc, p<0.001). In the recurrent glioma cohort, 65/81 patients transformed to grade III or IV; hypermutation was identified at transformation in 30/53 (57%) treated with TMZ. Hypermutation occurred in 31 patients all had received TMZ and 30/31 had developed transformed tumors. Analyzing by specimen, hypermutation was associated with transformation (bootstrapped logistic regression p < 0.001). After transformation to grade III disease (n=47), hypermutation was associated with diminished survival (HR 5.6, p=0.007), controlling for molecular subtype and age at diagnosis. Patients with transformation to glioblastoma (n=18) had poor prognosis regardless of hypermutation (p=0.53). Four cases of spinal dissemination were identified, all of whom had hypermutated gliomas. CONCLUSIONS: Somatic hypermutation is common in transformed, initially low grade IDH-mutant diffuse gliomas treated with TMZ. After anaplastic transformation, somatic hypermutation is associated with reduced survival, independent of molecular subtype. These data have implications for the management of newly diagnosed and recurrent DLGG, and indicate a potential role for immunotherapy.

PATH-30. RECONSIDERATION FOR POOR PROGNOSIS OLIGODENDROGLIAL TUMOR CASES BASED ON WHO2007 AND WHO 2016

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Mounting evidence suggests that oligodendroglial tumor is associated with a more favorable prognosis comparing with astrocytic tumor. Treatment strategy of our department is as follows 1) grade 2 oligodedroglioma with less than 90% resection are treat with post operative radiation following with RTOG 9802. 2)grade 3 anaplastic oligodendroglioma are treated with ACNU and radiation therapy. Instead of better prognosis of oligodendroglial tumor, there are several patients with unfavorable clinical course of malignant transformation. In this study, we retrospectively analyse clinical data of unfavorable oligodendroglioma cases diagnosed by WHO2007 and WHO2016. Overall 237 cases of newly diagnosed by WHO2007 in 2001 to 2014 were further analysed. Fetal cases were diagnosed as Oligodendroglioma(OL) 5, Oligoastrocytoma(OA) 3, Anaplastic oligodendroglioma(AO) 13 and Anaplastic oligoastrocytoma(AOA) 3 in WHO2007. These tumor were re-evaluated with WHO2016 (OL 120, AO 65, NOS 22). Fetal cases of oligodedroglial tumor were OD-mt 5, AO-mt 4, NOS 1 and the lest were all astrocytic tumor, Diffuse astrocytoma(DA)-mt 3, DA-wt 1, Anaplastic astrocytoma(AA)-mt 2 and AA-wt 7 cases. OS of all oligodendroglial tumor was not reached, PFS was 8.5 months in OD and not reached in AO. Median OS and median PFS were 57,1 and 40,1 months in OD fetal cases and 31,3 and 18,6 months in AO fetal cases. Five OD fetal cases were all partial removal and 3 were treated by post-operative therapy. One out of four AO fetal cases was total removal and all cases were treated with post-operative therapy. There were several poor prognosis oligodendroglial tumor instead of their genomic alteration such as 1p19q LOH and IDH-mt. Aggressive tumor resection may also alter the natural history of unfavorable oligodendroglial tumor.

PATH-31. GIANT CELL GLIOBLASTOMAS: ANALYSIS OF MISMATCH-REPAIR (MMR) PROTEINS EXPRESSION, POLIMERASE ε (POLE) MUTATIONS AND THEIR ROLE IN TUMOR IMMUNORESPONSE

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Giant cell glioblastoma (gcGBM) is a rare (<1%) variant glioblastoma (GBM), in younger patients. Unlike the IDH-wild type GBM, they have a better prognosis. Mutations in the POLE and in MMR genes accelerate tumorigenesis, generating in some tumours an ultra-mutated phenotype. The lack of proofreading activity generates production of neoantigens, recalling tumour infiltrating lymphocytes, and immune-checkpoint ligands exposition. Aim of our study was to investigate MMR proteins expression, POLE mutations, related checkpoint molecules and the tumor immune-microenvironment in a group of gcGBMs compared to IDH-wild type GBMs. We performed a molecular and immunohistochemical analysis on 60 primary gcGBMs. All tumours where characterized for EGFR, PTEN, p53, IDH1, MGMT status by immunohistochemistry and/or molecular analysis. We investigated MMR protein (MSH6, MSH2, PML2 and MLH1), PD-L1, CTLA-4 and CD28 expression by immunohistochemistry in gcGBMs and in a group of standard GBMs. POLE mutations have been studied by direct sequencing of exons encoding its exonuclease activity. Then we assess the immunological status investigating the presence of lymphocytic infiltrates, microglia and macrophages, by CD3, CD4, CD8, CD68, CD163, MHC class II and IBA1. All the results obtained have been related to clinical data. The median survival time was 21 months, with 4 patients long survivors (>5 years), higher than in the standard group. The main findings where partial loss of expression of MMR proteins (overall MSH2 and MSH6) on 30% of cases, mostly related to presence of inflammatory infiltrates, also showing CD28 immunostaining. Microglia IBA1+ was significantly present in patients with longer survival. Correlation with PD-L1 and CTLA4 was found only in 2 cases. POLE sequencing displays mutation F367S on 20% of cases. Our results show that gcGBMs are an histological variant with increased tendency to ultra-mutated phenotype with a better prognosis and suggesting these patients as candidates for immunotherapy.

PATH-32. BRAIN TUMOR CLASSIFICATION UPDATES FROM cIMPACT-NOW, THE CONSORTIUM TO INFORM MOLECULAR AND PRACTICAL APPROACHES TO CNS TUMOR CLASSIFICATION

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cIMPACT-NOW (the Consortium to Inform Molecular and Practical Approaches to CNS Tumor Taxonomy) was created to provide a forum to evaluate and recommend proposed changes to future CNS tumor classifications. While it is understood that the major impact on international brain tumor classification comes about through the WHO classification update process, it is anticipated that this additional process will see impact in selected tumor types and in time periods between the WHO classification updates. Over the past year, cIMPACT-NOW has convened three working committees (WC), each of which has focused on different classification issues. WC1 has debated the grading of diffuse gliomas relative to IDH status, and has formulated criteria for IDH-wildtype grade II and grade III diffuse astrocytic tumors that are likely to behave as glioblastomas, such as EGFR amplification and +7/-10 copy number changes. WC2 is developing a molecular classification of pediatric low-grade gliomas, focusing on the diffuse gliomas. WC3 has addressed miscellaneous issues, including clarifications of Not Otherwise Specific (NOS) and Not Elsewhere Classified (NEC) diagnoses and refined criteria for diffuse astrocytoma (e.g., the use of ATRX and p53 immunohistochemistry relative to 1p/19q testing) and diffuse midline glioma, H3 K27M-mutant. To date, two publications have resulted from WC3 and guidelines are expected soon from WC1 and WC2. The combined recommendations of the current cIMPACT-NOW WCs will be discussed in light of the WHO classification.

PATH-33. HEXOKINASE 2 KNOCKOUT VIA CRISPR REDUCES DOWNSTREAM GENE EXPRESSION, IMPLICATING A REDUCTION IN CELL PROLIFERATION AND DRUG RESISTANCE

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HK2 has a prominent role in aerobic glycolysis and has been implicated in many cancer types including GBM, with overexpression associated with drug resistant phenotypes. Previously, we have demonstrated HK2 expression was upregulated between 6 to >1000 fold in GBM biopsy tissue (n=100) and patient derived cell cultures (n=13), compared to normal brain tissue. In the

present study we have knocked out HK2 using CRISPR in patient-derived cultures (n=3) and the established cell line U251MG, to determine changes in the rate of cell proliferation and drug sensitivity. Additionally downstream expression changes were investigated via Qiagen profiler arrays, across 84 key genes involved in the regulation and enzymatic pathways of glucose metabolism. A substantial growth rate reduction between 38 to 44% (p<0.007) was demonstrated in CRISPR-modified cultures after 7 days compared to non-CRISPR cultures. Sensitivity to metformin was also significantly (p<0.0001) increased in response to HK2 knockout, where average ID50 values were 60% lower in cultures. Additionally CRISPR modified cultures yielded greater synergistic (CI<1) and additive effects (CI=1), with metformin and temozolomide combination treatment. Array data revealed an extensive change in downstream gene expression in CRISPR-modified cultures, between 25 to 48 genes were downregulated compared to the corresponding non-CRISPR cultures. Furthermore CRISPR-modified cultures demonstrated a similar reduction in downstream expression when compared to GBM biopsy tissue, conversely a greater number of genes had unchanged expression levels compared to normal brain tissue. This study demonstrates the predominant role of HK2 within the glycolytic pathway, with overexpression potentially key in driving the genetic alterations downstream. HK2 knockout revealed considerable ubiquitous reductions in downstream gene expression compared to GBM biopsy tissue and non-CRISPR cultures. Additionally an increase in drug sensitivity was depicted with the loss of HK2 signifying the potential of HK2 inhibition as a novel therapy in a significant subset of GBM.

PATH-34. VENTRICULAR-SUBVENTRICULAR ZONE CONTACT BY GLIOBLASTOMA IS NOT ASSOCIATED WITH MOLECULAR SIGNATURES IN BULK TUMOR DATA

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Whether patients with glioblastoma that contacts the stem cell niche of the ventricular-subventricular zone (V-SVZ+GBM) have a distinct survival profile from V-SVZ-GBM patients independent of other known predictors or molecular profiles is unclear. Using multivariate Cox analysis to adjust survival for widely-accepted predictors, hazard ratios (HRs) for overall (OS) and progression free (PFS) survival between V-SVZ+GBM and V-SVZ-GBM patients were calculated in 170 single-institution patients and 254 patients included in both The Cancer Genome (TCGA) and Imaging (TCIA) atlases. A multivariable analysis adjusted for age, Karnofsky performance score, IDH1 mutation, MGMT promoter methylation status, chemotherapy, radiation therapy, and extent of surgical resection revealed that V-SVZ contact was independently associated with decreased survival in both datasets (institutional patients: OS HR 1.55 [95% CI 1.03–2.33], P=0.037; PFS HR 1.57 [1.08–2.28], P=0.018; TCIA/TCGA patients: OS HR 1.69 [1.28–2.24], P<0.001; PFS HR 1.24 [0.91–1.7], P=0.18). Thorough TCGA molecular data analyses were conducted using differential molecular feature extraction, gene expression network construction, clustering methods, and dimensionality reduction. All analyses revealed that V-SVZ contact by GBM was independent of mutational, DNA methylation, gene expression, and protein expression signatures in the bulk tumor. Therefore, while survival of GBM patients is independently stratified by V-SVZ contact, with V-SVZ+GBM patients displaying a poor prognosis, the V-SVZ+GBMs do not possess a distinct molecular signature at the bulk sample level. Focused examination of the interplay between V-SVZ cytoarchitectural features, microenvironmental factors, and cancer cells within glioblastomas using subpopulation- or single-cell-based approaches is warranted.

PATH-35. FREQUENCY AND CHARACTERISTICS OF H3K27M-MUTATION IN ADULTS WITH RADIOGRAPHICALLY-DETERMINED MIDLINE GLIOMAS

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BACKGROUND: H3F3A mutations define the entity of Diffuse Midline Glioma, which was added to the WHO 2016 classification. There have been several reports describing the clinical, prognostic, and histopathological implications of this mutation. It is unclear, however, what proportion of adults with gliomas occurring in the midline have an H3 K27M mutation. We set out to define this in a single-institution, retrospective cohort study. METHODS: From 850 consecutive gliomas in adults we identified 163 cases with radiographically-determined midline gliomas (brainstem, thalamus, basal ganglia, corpus callosum, spinal cord, or cerebellum). Clinical cases were reviewed in accordance with IRB guidelines. FFPE tissue was obtained from 120 cases and stained for H3 K27M. RESULTS: A H3 K27M mutation was identified in 18 of 120 cases (15%). As compared to non-H3 K27M mutated tumor, average age was 45.1 ± 12.8 versus 53.1 ± 16.7 years (p=0.2). 56% were female (p=0.3). 83% had contrast enhancement on MRI (p = 0.79). All H3 K27M mutant tumors were WHO grade III or IV on histology, while non-mutant tumors encompassed all four grades (p = 0.08). The most common locations to have H3 K27M-mutated tumors were midbrain (2/2; 100%), pons (4/10; 40%), cerebellum (6/22; 27.3%), spinal cord (2/13; 15.4%), and thalamus (3/30; 10%). Median survival was 16 ± 6.0 months as compared to 8.1 ± 3.6 months in non-mutated midline high grade gliomas (p = 0.15). CONCLUSIONS: H3K27M mutated tumors are common in gliomas located along the midline and this molecular subtype should be considered in adults of all ages and grades with midline tumors, regardless of tumor location or contrast enhancement. Survival was not significantly different from non-H3 K27M mutated tumors, though a larger dataset will be necessary for confirmation.

PATH-36. IDH AND TERT PROMOTER MUTATIONS IN NON-DIAGNOSTIC BIOPSIES FROM GLIOMA PATIENTS

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BACKGROUND: Non-diagnostic biopsies are a recurrent issue in patients with a suspected brain tumor. Herein, in order to explore the utility of molecular testing in this setting, we determined whether IDH and TERT promoter (pTERT) mutations can be detected in non-diagnostic biopsies from glioma patients. METHODS: Using Snapshot PCR, we retrospectively assessed IDH and pTERT mutation status in 28 adult glioma patients in whom a first non-diagnostic biopsy had led to perform a second biopsy. RESULTS: Median age at diagnosis was 65 years and median delay between the first and second biopsy 21 days. The first biopsy consisted of not characterizable infiltrated glial cells (n=19), hemorrhage (n=4), necrosis (n=2) or normal tissue (n=3). The second biopsy demonstrated an IDH-wildtype glioblastoma (n=22), an IDH-wildtype astrocytoma (n=4), an IDH-mutant oligodendroglioma (n=1) and an IDH-mutant astrocytoma (n=1). A pTERT mutation was present in 21 cases. Retrospectively, the same IDH and pTERT mutations were identified in the non-diagnostic biopsies of the 2 patients with an IDH-mutant glioma and of 12 out of 21 patients with a pTERT-mutant glioma (57%). Overall an IDH and/or a pTERT mutation were detected in the non-diagnostic biopsies of 13 out of the 22 IDH-mutant and/or pTERT-mutant gliomas (59%) and in 13 out of the 28 cases included in the present series (46%). CONCLUSION: IDH and pTERT mutations can be detected in a high percentage of non-diagnostic biopsies from glioma patients supporting a role for molecular testing in the interpretation of non-diagnostic biopsies from patients with a suspected brain tumor.

PATH-37. LIQUID BIOPSY FOR IDENTIFICATION OF NEWLY DIAGNOSED GLIOMA

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INTRODUCTION: In patients with newly diagnosed intracerebral lesions based on MRI, gliomas are often suspected, but MRI is rarely definitive thus necessitating biopsy. For non-enhancing lesions involving eloquent or deep-seated structures, diagnosis can be especially challenging as biopsy may be relatively risky or undesirable to the patient. In this study, analysis of plasma isolated cell-free DNA and exosome mRNA and miRNA from newly diagnosed glioma patients and from cancer-free volunteers was used to predict disease. METHODS: Blood was drawn from 40 patients with newly diagnosed gliomas (28 high grade glioma (HGG), 12 low grade (LGG)) and 10 healthy volunteers without documented history of cancer. High quality DNA and RNA was isolated and sequenced using Next Generation Sequencing and Digital Droplet PCR was used for detection and verification of trace molecular artefacts. Multianalyte processing yielded data that was harmonized and interpreted through an Artificial Intelligence

based algorithm to assess for possible glioma and to assign grade. EGFRviii and IDH1 mutations were also analyzed and compared to molecular testing from tumor specimens. RESULTS: 97.5% (39 of 40) of glioma patients were deemed to have gliomas by plasma testing. 96% of HGG patients and 67% of the LGG patients were correctly graded. Of the 10 healthy controls, 8 were concluded to be cancer-free. Two of the patients were suspicious for malignancy, of which one was possible glioma. IDH1 and EGFRviii mutation had concordance at 74 % (26/35) and 59% (12/16), respectively. CONCLUSIONS: Analysis of plasma cell free tumor derived DNA and RNA was highly sensitive for detecting glioma with high agreement in grading as well. In patients with newly diagnosed intracerebral lesions, this may be a useful screening test to determine the need for more invasive testing, i.e. biopsy/resection. Further testing in blinded samples from brain tumor patients and healthy subjects will follow.

PATH-38. CORRELATION OF ALTERATION OF HLA-F EXPRESSION AND CLINICAL CHARACTERIZATION IN 593 BRAIN GLIOMA SAMPLES

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BACKGROUND: Human gliomas are highly fatal tumor with a significant feature of immune suppression. The immune system in glioma is gradually revealed, and immunotherapy is expected to improve the survival of glioma patients. With a deep understanding of the immune microenvironment of glioma, immunotherapy of gliomas has been increased exponentially in recent years. Searching for key regulators of immune response in glioma will provide clinical targets for immunotherapy. In our research, we focus on human leukocyte antigen (HLA) system, responsible for regulating the immune system, and discovered the relationship between HLA-F expression and clinical prognosis in gliomas. METHODS: A total of 593 gliomas patients are concluded in our research, 325 patients from Chinese Glioma Genome Atlas (CGGA) and 268 patients from GSE 16011 set. Kaplan-Meier (KM) analysis is performed to explore the prognostic value of HLA-F. T test analysis is used to find the distribution difference in various groups. R language packages are used for other statistical computations and figures drawing. RESULTS: HLA-F was significantly negatively correlated with overall survival (OS) in all grade gliomas and glioblastoma (GBM). Moreover, HLA-F was enriched in GBM and IDH1 wild-type group, and HLA-F was a mesenchymal subtype marker. Pearson correlation test showed that HLA-F was correlated with other HLA-I molecules. CONCLUSION: HLA-F expression was positively with malignant phenotype and negatively with OS indicating that HLA-F could predict the immune state in glioma, and might be a clinical target of glioma immunotherapy. Key Words: HLA-F; glioma; immunotherapy; OS

PATH-39. ASTROCYTOMA OF THE SPINAL CORD: A GENETIC CHARACTERIZATION AFTER MICROSURGICAL RESECTION

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INTRODUCTION: The revised version of the WHO classification system (2016) introduced molecular markers being of prognostic importance in gliomas. Primary spinal cord astrocytomas are very rare. Aggressive surgical resection is believed to be critical for extending progression free and overall survival. However, the prognostic significance of molecular variables remains unclear for these tumors. Herein we investigate molecular chances of spinal gliomas, which may allow more accurate risk stratification. METHODS: We performed genome sequencing in 10 spinal astrocytomas undergoing surgical resection between 2000 and 2017. These spinal astrocytomas include glioblastomas (WHO grade IV), anaplastic astrocytomas (WHO grade III), diffuse astrocytomas (WHO grade II) and pilocytic astrocytoma (WHO grade I). RESULTS: We identified 5 spinal glioblastomas, 1 anaplastic astrocytoma, 2 diffuse astrocytomas and 2 pilocytic astrocytomas. Median overall survival (OS) was 6 months (range: 2–14 months) for grade IV tumors, 33 months (range: 30–136 months) for grade II and III tumors and 95 months (range: 49–141 months) for grade I tumors, respectively. One grade II and one grade I tumor were carrying the IDH1 and IDH2 mutation, all other tumors were IDH wild type (OS: 93 vs. 10 months). Gross total resection was not achieved in any patient. 9 patients received adjuvant radiotherapy. The most current findings in spinal GBM were H3F3A mutations (5/5) and ATRX mutation (3/5). H3F3A mutation was observed in 1 WHO grade II tumor with a OS of 33 months. Mutation in H3F3A and WHO grade was associated with shortened OS in univariate analysis. WHO grade II tumors were found to have mutations in CCND2, DDX3X, EGFR, HIST1H3B, KIT, MYC, PDGFRA, PTCH2, SMARCA4 and TSC2. CONCLUSION: Genomic analysis of spinal astrocytomas provides an opportunity to identify potential clinically relevant information. These data indicate an association between H3F3A mutation and a shortened overall survival in spinal astrocytomas.

PATH-40. TARGETED NEXT GENERATION SEQUENCING (NGS) OF YOUNG ADULTS WITH ISOCITRATE-DEHYDROGENASE WILD-TYPE GLIOBLASTOMA (IDH-WT GBM) REVEALS NEGATIVE PROGNOSTIC IMPACT OF EPIDERMAL GROWTH FACTOR RECEPTOR AMPLIFICATION (EGFRAMP)

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BACKGROUND: Young adults with IDH-WT GBM represent a rare, understudied population compared to pediatric, IDH-mutant, or typical (elderly) GBM. We aimed to explore clinically detected genomic alterations in this population and their prognostic impact. METHODS: We identified patients ages 21 45 with newly diagnosed, previously untreated IDH-WT GBM whose tumors underwent NGS at our institution. Patients with hereditary cancer syndromes were excluded. The NGS panel detects pathogenic variants by targeted exome sequencing of 47 (2014–2016) or 153 (2016-present) genes. Clinical characteristics and overall survival (OS) were collected. These data were also collected from a contemporaneous cohort of older (>=65) patients with newly diagnosed, IDH-WT GBM. RESULTS: 28 young and 30 older patients were included. In young patients, 12 (43%) had an EGFR alteration [2 (7%) EGFR mutation, 7 (25%) EGFR amplification (EGFRamp), and 3 (13%) both EGFRamp and EGFRvIII]. Other mutations detected in 2 young patients were TP53 in 7 (25%), BRAF (V600E) in 3 (11%), RB1 in 3 (11%), PTEN in 2 (7%), SETD2 in 2 (7%), and DNMT3A in 2 (7%). Differences detected in older vs. younger patients were more frequent PTEN mutations (27% vs. 7%, p=0.049) and MGMT methylation (50% vs. 25%, p =0.06). In young patients, median OS was 19.5 months (95% CI 15.9–24.4), and EGFRamp was associated with inferior median OS (16.3 vs. 23.5 months, p=0.047). There was no difference in OS by EGFRamp in older patients. CONCLUSIONS: EGFRamp was associated with inferior OS in this contemporary cohort of young adults with IDH-WT GBM, whereas no association was detected in older patients. This suggests a potential role for targeting EGFR specifically in this population. In addition, consistent with prior studies, we found that MGMT methylation is less common in young patients with IDH-WT GBM, highlighting the need for alternatives to temozolomide.

PATH-41. PLASMA CELL-FREE DNA (cfDNA) CONCENTRATION IS INDEPENDENTLY ASSOCIATED WITH RADIOGRAPHIC TUMOR BURDEN IN NEWLY DIAGNOSED GLIOBLASTOMA (GBM) PRIOR TO INITIAL SURGICAL RESECTION

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BACKGROUND: Distinguishing between radiographic pseudoprogression and true tumor progression is challenging in patients with GBM. Recent data suggests that plasma cfDNA concentration may serve as a viable surrogate for tumor burden in other malignancies. We performed a pilot study to determine the feasibility of detecting cfDNA in patients with newly diagnosed GBM and explored its correlation with radiographic tumor burden and other relevant clinical variables. METHODS: We collected blood in Streck cfDNA tubes from patients with radiographically suspected high-grade glioma prior to planned initial surgical resection. Plasma was isolated using a 3-step centrifugation protocol. cfDNA was extracted from using a QIAamp Circulating Nucleic Acid Kit. cfDNA concentration (ng DNA/mL) was determined by quantitative real-time PCR (qPCR) for the ALU repeat element. Tumor burden was defined as the sum of products of diameters (SPD) of target enhancing lesions plus the SPD of the T2 FLAIR signal abnormality on preoperative MRI. RESULTS: 22 preoperative patients were enrolled and diagnosed histopathologically with GBM. The median cfDNA concentration was 10.9 ng/mL (IQR 7.2–23.6, range 0.48–37.6). There was a significant correlation between radiographic tumor burden and cfDNA concentration (Spearmans rho = 0.46, p = 0.03). In a multiple linear regression model, cfDNA concentration remained significantly associated with radiographic tumor burden (beta coefficient 1.87, p=0.03) after adjusting for age, sex, tumor Ki-67 proliferation rate, weight, and glomerular filtration rate (GFR). GFR was also independently (negatively) associated with cfDNA concentration. CONCLUSIONS: In this small pilot study, we demonstrated that plasma cfDNA is easily detected and quantified in patients with newly diagnosed GBM prior to initial surgical re-

section. In addition, plasma cfDNA concentration was independently associated with radiographic tumor burden. A prospective, longitudinal study monitoring cfDNA dynamics during standard treatment for GBM, including gold standard tissue correlates for tumor progression vs. pseudoprogression, is ongoing at our institution.

PATH-42. EGFR-AMPLIFIED IDH-WILDTYPE GLIOBLASTOMAS SELDOM TRANSFORM INTO A HYPERMUTATED PHENOTYPE

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INTRODUCTION: Current efforts to improve patient survival in recurrent glioblastomas (GBMs) are often based on targeting the tumors acquired genetic changes. However, most molecular data is derived from the initial tumor: resections of recurrent GBMs are seldom performed. This study aims to assess whether genetic traits of initial GBMs are still present at recurrence. METHODS: DNA/RNA was isolated from pairs of initial and recurrent Stupp-treated GBM tumor samples (FFPE) and sequenced on a panel of 365 cancer genes. MGMT promotor methylation was determined by MS-PCR, EGFRvIII by RT-PCR and TERT-promotor mutations by SNaPshot. RESULTS: 276 patients from ten medical centers in six countries were identified with median age of 54.1 years. Median survival was 23.7 months, and median time to second surgery 13.0 months. Only 10 of 186 sequenced matched tumor pairs were IDH-mutated (5.4%). Overall, genetic traits of initial GBMs were stable with a median retention rate of coding mutations of 81.8%. Similarly, copy number changes (CNVkit/GISTIC) generally were retained at tumor recurrence. However, the retention rate was also ~80% in all of the major GBM driver pathways (TP53 signaling, RAS-RAF-MEK-ERK, RTK signaling). This is important as decreases 20% of loss of a marker requires a doubling of the number of included patients to achieve a similar power (assuming an objective response rate of 40% as a positive outcome). One noticeable change was a loss of TERT-promoter mutations in 7.5% of recurrent samples. Only four tumors were hypermutated (> 100 coding mutations) at recurrence, though more samples (n=12) acquired mutations in MSH2 and MSH6. Only one EGFR-amplified tumor was hypermutated. CONCLUSION: EGFR-amplified GBMs seldom transform into hypermutated tumors which suggests immune-checkpoint inhibitors have limited clinical use in recurrent GBMs. Re-sampling should be considered prior to inclusion in targeted therapy trials to ensure proper patient selection.

PATH-43. RETROSPECTIVE RECLASSIFICATION OF ADULT GLIOMAS FROM A MANITOBA PATIENT COHORT ACCORDING TO THE WHO 2016 GUIDELINES

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The new WHO 2016 classification of CNS tumours classifies adult gliomas based on the presence or absence of two key molecular markers, IDH mutation and 1p/19q co-deletion. We applied the new criteria to Manitoba glioma patients diagnosed between 2013–2016 with the objective of assessing the distribution of molecular markers among the entire population of glioma patients, assess the prognostic power of the molecular markers, and evaluate the impact of the new classification system on patients. In total, 203 patients were included, and the majority of gliomas were IDH wild type (81.8%). Among the IDH mutant tumours, 9.9% were 1p/19q intact and/or ATRX loss, and 8.4% were 1p/19q co-deleted. The WHO 2016 was impactful on patients such that roughly one third were reclassified into 2016 diagnostic categories with different prognoses. Molecular markers were marginally better than grade at predicting progression free survival, however, the predictive power of the model was improved when molecular markers and grade were included in the same model.

PATH-44. THE LANDSCAPE OF SOMATIC MUTATIONS AND COPY NUMBER ALTERATIONS IN PRIMARY GLIOBLASTOMA IN JAPAN

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INTRODUCTION: Some differences in the frequencies of cancer genetic alterations across the population have been reported, including EGFR mutations in lung cancer. However, little information is provided in glioblastomas. Here, we investigated the mutational landscape of primary glioblastomas of Japanese population and its trends against the Cancer Genome Atlas (TCGA) dataset. METHOD: We retrospectively analyzed somatic genetic alterations of 220 cases diagnosed as primary glioblastoma based on CNS WHO2016, which were resected in Japan between December 2006 and November 2017. Mutational status of IDH1/2, TERT, and TP53 were determined using the Sanger technique. MGMT promoter methylation status was analyzed by quantitative methylation-specific PCR. Copy number alterations of eight major genes including EGFR were evaluated by using multiplex ligation-dependent probe amplification. The frequency of each alteration was compared with that in TCGA cohort. The relationships and survival impact of each molecular marker were also investigated. RESULTS: Our cohort showed lower frequencies of TERT mutation (56.8%), EGFR gain (55.0%), PTEN loss (44.1%), CDK4 gain (14.1%), and MDM2 gain (9.5%) when comparing with the data of TCGA. On the other hand, the frequencies of TP53 mutation (39.1%) and loss (40.4%) were higher. TERT mutation had a significant positive correlation with EGFR gain ($p < 0.001$) and PTEN loss ($p < 0.001$). In survival analysis excluding tumors with IDH mutation, unmethylated MGMT promoter and TERT mutation were significant independent prognostic factors, while other genetic aberrations were not. DISCUSSION: The frequencies of several genetic aberrations differed between Japan and TCGA cohort. Although further studies are needed, our results suggest that the racial differences in genetic alterations may affect the interpretation of clinical trials conducted in different countries.

PATH-46. NEURONAL DIFFERENTIATION IS INDUCED BY Gli3 IN WNT- AND SHH- ACTIVATED MEDULLOBLASTOMA

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BACKGROUND: We have previously investigated the expression of Gli3, a downstream target of the Sonic Hedgehog pathway, which main function is to suppress Gli1/2 in medulloblastomas. We found that Gli3 is associated with neuronal and glial differentiation in desmoplastic / nodular (D/N) type medulloblastomas (Miyahara et al., Neuropathology, 2013). In the present study, we investigated the expression of Gli3 in the molecular subgroups. METHOD: Thirty-one medulloblastomas treated at Department of Neurosurgery, Niigata University between 1982 and 2013 were retrospectively studied. Molecular classification into 4 subgroups (WNT-activated, SHH-activated, Group 3 and Group 4) was performed using Nanostring. HE and NeuN, GFAP, beta-catenin, GAB-1, and YAP-1 immunohistochemistry was performed. Furthermore, Gli3 and

Gli1 expression in each molecular subgroup was assessed in the public data base R2. RESULTS: Nanostring was considered reliable (confidence > 0.9) in 28 cases. Four cases were classified as WNT- activated, 5 cases as SHH-activated, 4 cases as Group 3 and 16 cases as Group 4. Gli3 was positive in 7 out of 9 (78%) WNT-/SHH- cases, whereas Gli3 was positive in only 8 out of 19 (42.1%) non-WNT-/SHH- subgroup (p = 0.1145, Fishers exact test). R2 database analysis confirmed that Gli3 was significantly elevated in WNT- and SHH-activated medulloblastoma. Gli1 was elevated in SHH-activated cases but suppressed in WNT-activated cases. IHC analysis revealed that Gli3 was elevated inside the nodules of D/N type medulloblastoma. Neuronal differentiation was seen in these nodules. CONCLUSION: These results suggest that Gli3 is elevated inside the nodules of SHH-activated medulloblastoma, whereas in WNT-activated cases, Gli3 suppresses HH signaling in the entire tumor.

PATH-48. FUSION TESTING IN ADULT VERSUS PEDIATRIC LOW AND HIGH GRADE BRAIN TUMORS FOR ELIGIBILITY FOR TRIALS

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BACKGROUND: Since January 2017, our molecular laboratory has assessed 47 pediatric and 23 adult primary brain tumors for fusions that might result in novel treatment; adults with glioblastoma (GBM) had been screened specifically for the STARTRK-2 study. This trial has open arms for patients whose tumors demonstrate ROS1, ALK, or NTRK 1/2/3 fusions. We now review our results in pediatric versus adults. MATERIALS AND METHODS: Fusion analysis was performed with the Archer FUSIONPlex Solid Tumor Kit, 1/2017-5/2018. RESULTS: 18 adults with GBMs (17/18 IDH-wildtype) screened for the STARTRK-2 study were negative for fusions or potentially oncogenic transcripts in ALK, ROS1 or MET. However, one adult IDH-wildtype GBM showed a FGFR3-TACC3 actionable fusion that might allow erdafitinib therapy. Three more were found to have fusions of uncertain significance: an IDH-wildtype GBM with EGFR-SEPT14 fusion, an IDH-wildtype GBM with EGFR-PSPH fusion, and the sole IDH-mutant GBM with ZMIZ1-FGFR2 fusion transcript. 5 additional adults were screened for BRAF V600E mutation / BRAF-KIAA1549 fusion, of which 1 fusion and 1 mutation was identified. 42 pediatric patients with low-grade glial/glioneuronal tumors were assessed: 10 had mutation and 5 fusion, potentially allowing for BRAF or MEK inhibitors. Actionable fusions for potential trial entry were found in a 6-year-old male with pilocytic astrocytoma (PA) (GOPC-ROS1 fusion), a 4 month-old male with congenital GBM (MZT2B-ALK fusion), and a 3 year-old female with epithelioid GBM (ETV6-NTRK3 fusion). FGFR1-TACC1 fusions were found in three pediatric patients (16-year-old female with PA, 9-year-old male with extraventricular neurocytoma, 1-year-old male with spinal cord PA). CONCLUSIONS: Actionable fusions can be found in both adult and pediatric patients, although STARTRK-2 study entry criteria were not met in any of the 18 adults. The most unusual fusions thus far have been the congenital GBM (MZT2B-ALKfusion) and the epithelioid GBM (ETV6-NTRK3 fusion).

PATH-49. GENOMIC ATTRIBUTES OF TUMOR EVOLUTION AND TREATMENT RESPONSE IN DIFFUSE GLIOMA

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INTRODUCTION: Though the genomic landscape of primary gliomas has been well characterized by The Cancer Genome Atlas, the genetic determinants of malignant transformation and response to therapy remains poorly understood. METHODS: Prospective clinical sequencing was performed on 1,004 gliomas from 923 patients. This dataset includes primary and recurrent tumors and contains detailed clinical annotation, including review of the patients imaging. RESULTS: We investigated the germline and somatic attributes of IDH1/2-wildtype and IDH1/2-mutant tumors at the time of diagnosis and recurrence. 13% of patients harbored either a pathogenic or likely pathogenic germline mutation, whereof 29% arose in genes mediating DNA repair. In astrocytomas, agnostic of IDH status, cell cycle alterations were depleted in low-grade tumors. Moreover, mutations in effectors of the cell cycle were associated with the development of enhancing disease in IDH-mutant astrocytomas but not oligodendrogliomas. IDH-mutant astrocytomas with a cell-cycle alteration have a significantly shorter progression-free survival from recurrence compared to tumors without a cell cycle alteration (median 2.5 vs. 35.3 months, HR 3.25, log-rank p-value 0.00061). Based on our data, hypermutation appears to occur exclusively in the context of pre-existing cell cycle alterations in astrocytic tumors, regardless of IDH status. We next correlated molecular findings with clinical behavior and treatment response and defined subsets of gliomas that are uniquely susceptible to targeted treatment and have a differential prognosis. CONCLUSION: Cell-cycle alterations are lineage-specific alterations associated with aggressive disease in glioma. Targeted genomic sequencing can identify subsets of tumors with a greater sensitivity to treatment and a better prognosis.

PATH-50. HIGH DETECTION RATE OF MYD88MUTATIONS IN CEREBROSPINAL FLUID FROM PATIENTS WITH CENTRAL NERVOUS SYSTEM LYMPHOMAS

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OBJECT: Biopsy is generally necessary for the diagnosis of primary central nervous system lymphoma PCNSL. However, surgical biopsy has problems of fatal hemorrhagic complication and false negative findings, so more safe and reliable diagnostic methods are required. The aim of this present study is to detect MYD88 mutations, an important driver mutation, in cerebrospinal fluid (CSF) taken from PCNSL patients. METHOD: Fifteen CNS lymphoma (13 PCNSL, 3 CNS relapse from systemic lymphoma) patients were studied. We obtained cell free DNA (cfDNA) from CSF by lumbar puncture. CfDNA was extracted from 1ml of CSF, and both direct sequence and droplet digital PCR (ddPCR) were performed. Furthermore, we performed direct sequence from surgical obtained formalin fixed paraffin embedded (FFPE) tissue and analyzed the relationship to clinical data. RESULT: The mean cfDNA concentration of 1 ml CSF was 188.8 ng/ml [95% CI 124.2–253.8 ng/ml]. MYD88 mutations from CSF were detected in 60.0% (9 of 15 cases), and L265P in exon5 was the most frequent mutation in 8 out of 9 (88.8%) cases, S219C in exon3 was detected in one case. In 2 patients, MYD88 mutation was confirmed by ddPCR but not direct sequence. In all 8 cases with sufficient FFPE tissue for DNA analysis, MYD88 mutation was confirmed in the FFPE tissue. In 1 patient, there was insufficient FFPE tissue for DNA analysis. MYD88 mutation was not detected in the CSF of all 6 patients without MYD88 mutation in the tumor. CONCLUSION: This pilot study provided evidence that the somatic driver mutation MYD88 can be reliably detected by direct sequence and ddPCR in the cfDNA taken from 1 ml of CSF in patients with CNS lymphomas.

PATH-51. DNA COPY NUMBER PROFILING ACROSS GLIOBLASTOMA POPULATIONS HAS IMPLICATIONS FOR CLINICAL TRIAL DESIGN

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BACKGROUND: Copy number alterations form prognostic molecular subtypes of glioblastoma with clear differences in median overall survival. In this study, we leverage molecular data from several glioblastoma cohorts to define the distribution of copy number subtypes across random cohorts as well as cohorts with selection biases for patients with inherently better outcome. METHODS: Copy number subtype frequency was established for four glioblastoma patient cohorts. Two randomly selected cohorts include The Cancer Genome Atlas (TCGA) and German Glioma Network (GGN). Two more selective cohorts include the phase II trial ARTE in elderly patients with newly diagnosed glioblastoma and a multi-institutional cohort focused on paired resected initial/recurrence glioblastoma. The paired initial/recurrence cohort also had exome data available, which allowed for evaluation of multidimensional scaling analysis. RESULTS: Smaller selective glioblastoma cohorts are enriched for copy number subtypes that are associated with better survival, reflecting the selection of patients who do well enough to enter a clinical trial or who are deemed well enough to undergo resection at recurrence. Adding exome data to copy number data provides additional data reflective of outcome. CONCLUSIONS: The overall outcome for diffuse glioma patients is predicted by DNA structure at initial tumor resection. Molecular signature shifts across glioblastoma populations reflect the inherent bias of patient selection towards longer survival in clinical trials. Therefore it may be important to include molecular profiling, including copy number, when enrolling patients for clinical trials in order to balance arms and extrapolate relevance to the general glioblastoma population.

PATH-52. UTILIZING NEXT GENERATION SEQUENCING REPORTS IN CLINICAL DECISION MAKING: REPORT FROM THE NATIONAL INSTITUTES OF HEALTH (NIH) NEURO-ONCOLOGY BRANCH (NOB) NATURAL HISTORY STUDY (NHS) PRIMARY BRAIN TUMOR PANEL (PBTP)

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BACKGROUND: The use of molecular diagnostics is an integral component of CNS cancer care. The NIH NOB PBTP includes 80 alterations (55 mutations/copy number variations and 25 gene fusions) identified as relevant to the primary CNS patient population. We have previously reported on the diagnostic utility. Identified therapeutic target results from the PBTP are reported here. METHODS: PBTP results from patients enrolled on the NOB NHS are reported. Targetable alterations were defined using the NCI MATCH trial list. The proportion of cases with identified alterations based on recognized diagnostic histology and recurrence are reported. RESULTS: 190/282 (67%) PBTPs reported significant alterations; 132 from initial diagnosis and 58 recurrent samples;10 had two sequential samples analyzed. Astrocytoma, grade 2–4 (126/190, 66%), was the most common histologic tumor type. Over 60% of cases had a unique alteration profile, appearing only once in the data set. At diagnosis 74% had 1–3 alterations. At recurrence 45% had >3, and 9% had > 7 alterations. New and increasing alterations were seen in 80% (8/10) of sequential cases, with 1 case showing an entirely different alteration pattern. Targetable alterations were identified in 87/190 (46%) PBTP results. Exclusively in astrocytomas, EGFR (19%), and CDK4/CDK6 (12%) were found. Less frequent were: BRAF (5%) (8 astrocytomas, 2 pleomorphic xanthoastrocytoma), FGFR (3%) (5 GBM, 1 rosette-forming glioneuronal tumor), MET (3%) (3 GBM, 1 anaplastic oligodenroglioma, 1 anaplastic ependymoma), PTCH1 (3%) (3 astrocytoma, 1 ependymoma, 1 medulloblastoma), NTRK (2%) (2 astrocytomas, 1 oligodendroglioma). CONCLUSIONS: The PBTP has demonstrated diagnostic and therapeutic utility, with actionable targets found in nearly half of analyzed samples. The profile and number of alterations was higher at recurrence, underscoring the need for contemporary resampling and analysis. Importantly, 2/3 of the results showed a unique alteration profile highlighting the unique nature of each patients tumor.

PATH-53. CEREBROSPINAL FLUID TARGET DEEP SEQUENCING IMPLICATES AN ALTERNATIVE ASSAY OF GLIOBLASTOMAS DRIVER GENES IN THE CLINICAL SETTINGS

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AIM: The goal of the current study was to explore whether routine lumbar puncture and high-throughput sequencing of CSF could better identify tumor-driving mutations both contribute in glioblastomas development in patients and provide clinically meaningful insights into the driver genes of glioblastomas and their treatment response. METHODS: We characterized genomic architecture and implicated mutation levels differ between cerebrospinal fluid (CSF) and plasma samples collected 7 days of clinical follow-up using targeted deep sequencing. We sequenced coding region, exon-intron boundaries and UTRs of 50 central nervous system cancer-associated genes in cell-free DNA from CSF obtained through routine lumbar puncture in 5 patients with Glioblastomas. RESULTS: Our results demonstrated that serial changes in circulating levels of important driver mutations correlate with different prognosis of the cohort. The comparison of CSF and plasma samples in each single patient with Glioblastomas shows that circulating tumour DNA in CSF better reflect the sequential change of the driver genes than that in plasma. CONCLUSIONS: Cerebrospinal fluid target deep sequencing implicates an alternative assay of glioblastomas driver genes in the clinical settings.

PATH-54. UTILITY OF NEXT GENERATION SEQUENCING IN ADULT PRIMARY BRAIN TUMORS: IMPACT ON DIAGNOSIS AND PERSONALIZED THERAPY

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BACKGROUND: Historically, the molecular characterization of gliomas and other brain tumors has been controversial. A diagnosis was primarily made by histological analysis alone, despite concordance among neuropathologists being as low as 52% in some cases. Based on the 2016 World Health Organization guideline update, molecular profiling of brain tumors is now considered best practice for central nervous system tumors. In addition to providing a more accurate diagnosis, next generation sequencing of brain tumors can identify molecular alterations amenable to targeted therapy. METHODS: A retrospective chart review of 251 adult primary brain tumor patients with next generation sequencing performed was conducted at a single institution. Comparison of histological and molecular diagnosis, identification of potentially actionable alterations, and treatment choice based on alterations was analyzed. RESULTS: Clarification of diagnoses was observed in gliomas, but sequencing did not alter diagnosis in ependymomas, meningiomas, or medulloblastomas. Excluding ependymomas, there were 220 gliomas, of which 33 cases (15%) had clarification of the diagnosis. Of these 33 cases, 18 instances had clarification of IDH mutation status and the remaining 15 had a different or narrowed diagnosis based on the molecular features. Potentially actionable molecular alterations were found in 60% (150/251) of patients including *CDKN2A/B, CDK4, EGFRvIII*, *BRAF, BRCA2, SMO, PTCH1*, hypermutator genes (*MSH2, MSK6, PMS2, MLH1*), and increased mutation burden. Patients were treated with therapies targeting at least one actionable alteration in 9% of all cases (22/251). CONCLUSIONS: This study validates next generation sequencing as a clinical tool to obtain an accurate diagnosis and identify targetable alterations in brain tumor patients which have an impact on treatment decisions. These data support that integration of this technology will be essential in clinical trials exploring personalized therapy in neuro-oncology patients.

PATH-55. CLINICAL AND MOLECULAR RECURSIVE PARTITIONING ANALYSIS OF HIGH-GRADE GLIOMA TREATED WITH IMRT

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INTRODUCTION: Despite multimodal treatment for high-grade gliomas, prognosis remains grim. Since the development of the RTOG-RPA, high-grade gliomas have seen the widespread introduction of temozolomide and tumor onco-genetics. We aimed to determine whether the RTOG-RPA retained prognostic significance in the context of modern treatment paradigm, as well as generate an updated RPA incorporating both clinical and genetic variables. METHODS: Patients with histologically-proven high-grade gliomas treated with IMRT between 2004–2017 were reviewed. The primary endpoint was overall survival from date of diagnosis. Primary analysis compared actual survival rates to that expected of corresponding RTOG-RPA class. Secondary analysis utilized the rpart function to recursively partition overall survival by numerous clinical and genetic pre-treatment and treatment-related variables. A tertiary analysis recursively partitioned a subset of patients in which the status of all genetic markers were known. RESULTS: We identified 878 patients with a median overall survival of 14.2 months (95% CI: 13.1–15.3). Our cohort validated the relative prognostic ordering of the RTOG RPA survival classes except class II. Our new RPA created 7 significantly different survival classes (p2 = 584) with median survival ranging from 96.4 to 2.9 months based on age, histology, MGMT methylation, radiation fractions, tumor location, radiation dose, temozolomide, and resection. Our second RPA of our genetic subset (291 patients) generated 5 significantly different survival classes (p2 = 166) with survival ranging from 65.3 to 5.6 months based on age, IDH1 mutation, MGMT methylation, neurologic functional classification, IMRT hospitalization, temozolomide, and KPS. CONCLUSION: This series represents a large RPA analyzing clinical and genetic factors and generated 7 distinct survival classes. Further assessment of patients with fully available genetic markers generated 5 distinct survival classes. These classifications need to be validated by a prospective dataset and compared against the RTOG-RPA to determine if they provide improved prognostic power.

PATH-56. CHEK2 MUTATION IN HIGH-RISK MEDULLOBLASTOMA

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Childhood cancer remains the leading cause of non-accidental death in children, with brain and central nervous system malignancies comprising about 18% of new diagnoses each year. Combination therapy, including surgery, chemotherapy and radiation therapy in standard-risk medulloblastoma has led to cure rates of 80% or better. The 20% of tumors that do not respond to traditional therapies, or relapse early, could be due to activation

of tumor predisposition genes. CHEK2 is cell cycle checkpoint kinase 2, a tumor suppressor gene associated with increased risk of malignancies, most commonly breast and prostate cancer. Here we discuss a case of medulloblastoma, with evidence of germline and tumor CHEK2 mutation. This 4 year-old child was initially treated with surgery, achieving gross total resection, chemotherapy, tandem stem cell rescue, as well as local radiation therapy to posterior fossa. One year after completion of therapy, she relapsed with disease outside of the radiation field. Next generation sequencing of the whole genome performed on tumor tissue resulted in CHEK2 T367 mutation in the absence of TP53 mutation, or other mutations of known tumor suppressor genes. TP53 mutation is a cancer predisposition syndrome, associated with Li Fraumeni syndrome. CHEK2 mutation has been associated with worse progression-free survival in cases of non-Hodgkin's lymphoma. Thus far, there have been no reported primary brain and central nervous system malignancies associated with CHEK2 mutation. This finding of CHEK2 mutation in medulloblastoma further supports the need for next generation sequencing on high-risk tumor types to predict response to therapy and overall prognosis.

PATH-57. MOLECULAR DETERMINANTS OF RECURRENCE AND MALIGNANT TRANSFORMATION IN DIFFUSE LOW GRADE GLIOMA

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INTRODUCTION: Tumor recurrence and malignant transformation (MT) are the main contributing factors of poor prognosis in treating diffuse infiltrative low grade gliomas (LGG). In the current study, we conducted an integrated approach – transcriptomic analysis and in-depth clinical and molecular information to find susceptible genes associated with MT. MATERIALS AND METHODS: From 2004 to 2015, a total of 56 newly diagnosed World Health Organization (WHO) grade II LGG tumor samples were collected during surgical resection. All tissues were subjected to whole exome sequencing (WES) as well as transcriptome profiling by RNA sequencing (RNA-Seq) analysis. RESULTS: Twenty-eight (50%) patients had disease recurrence with a median RFS of 47.6 months. Among 18 of the 28 (64.3%) re-operated patients had MT with a median malignant transformation-free survival (MTFS) of 82.3 months. After adjusting all clinical factors in multivariate Cox proportional hazard model, male gender was the only clinical risk factors of poorer RFS with an adjusted hazard ratio (AHR) of 3.695 (95% CI: 1.353–10.093, p=0.0108); larger tumor volume (AHR:5.422, 95% CI: 1.549–18.977, p=0.0082) was the only risk factor of shorter MTFS. Of the top 50 expressed variant genes, we identified 15 genes that could cluster the 56 samples differentially into two categories: over-expression (n=26) and under-expression (n=30). A further comparison of clinical characteristics between under-expression group and over-expression group revealed a significantly shorter RFS (39.6 vs. 82.6 months, p=0.0201) and shorter MTFS (59.1 months, p=0.0239). Tumor locations in the paraventricular zone (PVZ) were significantly higher in the under-expression group (p=0.0466). CONCLUSIONS: Among molecular parameters, we found that down-regulation of a 15-gene signature was significantly correlated with tumor infiltrated to PVZ and with both shorter RFS and MTFS. Further examination of this prognostic gene signature reveals a functional connection to core neuronal processes, such as neurotransmission and calcium signaling.

PATH-58. MISMATCH REPAIR DEFICIENCY (MMRd) IN GLIOMA PATIENTS (PTS): FREQUENCY AND CORRELATION WITH CLINICAL, HISTOLOGICAL AND MOLECULAR CHARACTERISTICS

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BACKGROUND: Immunecheckpoint inhibitors (ICI) represent a new approach in oncology. The DNA MMRd would seem to be a predictor of ICI efficacy. We analyzed MMRd frequency in glioma PTS and its correlation with clinical, histological and molecular characteristics. METHODS: From July 2017 to May 2018, we prospectively analyzed glioma PTS for the presence of MMRd by immunohistochemistry (IHC): MSH2,MSH6,PMS2,MLH1. Clinical, histological and molecular characteristics were recorded. Chi-square test was used for analyzing their correlations with MMRd. RESULT: 167 PTS enrolled: 78% glioblastoma (GBM),14% anaplastic astrocytoma (AA),1% ependymoma,2% anaplastic oligodendroglioma (OD) and 5%LGG. The analyses were assessed on samples of first (82%) and second surgery (18%). 134 PTS analyzed for IDH status: 99 IDH wt; 117 for MGMT: 68 methylated. 27PTS (16%) showed MMRd by IHC (MSH2 in 48%, MSH6 in 55.6%, PMS2 in 18.5% and MLH1 in 14.8%): 33% of AA, 14% of GBM, 33% of OD and 0% of LGG (p=0.2). MMRd was found in 13% and 32% on first and second surgery samples (p=0.03). PD-L1 expression analysis was performed in 60 cases: no expression in 58%, ≥1%and<50% in 38%; ≥50% in 10%. MMRd was not correlated with PD-L1 expression (p=0.3). MMRd was found in 10% and 29% of IDHwt and IDHmut gliomas(p=0.008); MMRd was showed in 10% and 21% of PTS with unmet and metMGMT(p=0.1). Among MMRd tumors,7 were also investigated by molecular analysis (PCR) of mononucleotide markers: in only 1PT (14%) was confirmed MMRd in agreement with IHC analysis (p=0.1.). CONCLUSIONS: We showed a small group of glioma PTS have MMRd by IHC, expecially at second surgery. Correlation was observed between IHC MMRd and IDH mutational status. No association was demonstrated between IHC MMRd and histology, MGMT status, PD-L1 expression or molecular analysis of MMRd. A prospective study analyzing ICI efficacy in MMRd PTS should be warranted.

PATH-59. THALAMIC GLIOMAS WITH H3 K27M MUTATION. A CASE SERIES AND LITERATURE REVIEW

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OBJECTIVE: To report 3 cases of thalamic gliomas harboring H3 K27M mutation and review the available literature. BACKGROUND: Thalamic gliomas are rare tumors. They predominantly occur in children and young adults. The World Health Organization 2016 classification of brain tumors defines diffuse midline glioma H3 K27M-mutant as a new entity and includes diffuse intrinsic pontine glioma (DIPG) among gliomas in other midline locations such as thalamus and spinal cord. Previously published case series report an unfavorable outcome in patients harboring this mutation. METHODS: Retrospective chart reviews were conducted for patients with thalamic gliomas harboring H3 K27M mutation at the University Of Texas Southwestern Medical Center. 3 patients with thalamic gliomas were identified.The patients consented to publication of their diagnostic studies. RESULTS: 2 were female and 1 was male. Age at diagnoses was 18, 21 and 24. All 3 patients presented with headaches secondary to increased intracranial pressure. Gadolinium enhanced brain magnetic resonance imaging in all 3 patients showed thalamic cysts with enhancing mural nodules. 2 out of 3 patients underwent extensive resection of the mass and one underwent a subtotal resection. Pathology demonstrated WHO Grade I pilocytic astrocytoma in 2 cases and high grade astrocytoma in the third. All 3 samples were positive for the H3 K27M mutation. Due to the presence of the H3 K27M mutation, all 3 patients were treated as WHO Grade IV Glioblastoma with chemoradiation and maintenance chemotherapy following resection despite 2 patients demonstrating a pilocytic astrocytoma for which complete resection is curative. CONCLUSION: In children and young adults, gliomas harboring the H3 K27M mutation are characterized by a midline location and unfavorable prognosis. These cases highlight the need to aggressively treat midline gliomas with H3 K27M mutation regardless of their histological appearance. New targeted therapeutic approaches are needed to treat these tumors.

PATH-60. BIOINFORMATIC PROFILING IDENTIFIES THE SECRETED GLYCOPROTEIN ADAMTSL4 TO BE A POTENTIAL NOVEL IMMUNE-RELATED BIOMARKER FOR PRIMARY GLIOBLASTOMA

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BACKGROUND: Research on immunotherapy of glioblastoma (GBM) has been increasing exponentially in recent years. As a targeted therapy, series of biomarkers have been identified in the local tumor tissue, while circulating makers which could be detected in the body fluids are still blank. ADAMTSL4, a secreted glycoprotein, was earlier found to contribute a lot in a GBM prognostic signature. We aimed to investigate the role of ADAMTSL4 at transcriptome level and its relationship with clinical practice in primary GBM. METHODS: A cohort of 88 primary GBM patients with RNA-seq data from the Chinese Glioma Genome Atlas (CGGA) was analyzed, and 168 pGBM patients from TCGA were included as validation. Multiple bioinformatic tools and predictive models were applied to investigate the ADAMTSL4-associated immune microenvironment sta-

tus. RESULTS: We found that ADAMTSL4 was enriched in GBM, IDH wild-type and MGMT methylated groups. According to the TCGA classification scheme, ADAMTSL4 can act as a potential marker for malignant subtypes. Bioinformatical analyses revealed that ADAMTSL4 was significantly correlated to the immune processes in GBM, especially representing the infiltration of immune cells and complicated tumor microenvironment. Clinically, high expression of ADAMTSL4 was an independent indicator of poorer prognosis. CONCLUSION: The expression of ADAMTSL4 is closely related to the clinicopathologic characteristics of pGBM. Meanwhile, it plays a significantly predictive role in immune processes. For its characteristic of secreted glycoprotein, ADAMTSL4 is a promising circulating biomarker for pGBM, deserving further investigation.

PATH-61. A NOVEL ANALYSIS MODEL OF MGMT METHYLATION PYROSEQUENCING OFFERS AN OPTIMAL PREDICTIVE PERFORMANCE IN GLIOMAS

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The *MGMT* methylation is crucial for clinical decision-making in gliomas. *MGMT* pyrosequencing results were often dichotomized based on a threshold value for the average methylation of several tested CpGs. However, the frequent 'gray zone' results of this method immersed physician in a dilemma, and a novel analysis model which could address this issue is urgently required. In this study, we confirmed the *MGMT* promoter CpGs heterogeneity in 213 high-grade gliomas from two experimental cohorts, which included the seven-site cohort and the eight-site cohort with CpGs 72–78 and CpGs 75–82 tested, respectively. The optimal cut-off value of the methylation status for different CpGs also varied from 4% to 16%. Thus, we raised a novel analysis model, which comprehensively considered each individual CpGs methylation status with its own cut-off value determined by ROC, and the novel analysis defined MGMT promoter methylation when ≥ 3 CpGs exceeded their respective optimal cut-off values. Then we evaluated the predictive accuracy of the novel analysis model in 135 patients received temozolomide from the two experiment cohorts. The results were also validated in an independent cohort including 65 patients, and further compared with the methylation-specific PCR (MSP) approach. In all three cohorts, the novel analysis for CpGs 75–78 accurately predicted the therapeutic prognosis of patients whose methylation levels in the 'gray zone', and improved the AUCs from (0.67, 0.76, and 0.67) to (0.70, 0.84, and 0.72), respectively. Moreover, the advantages of the novel analysis were demonstrated regardless of WHO grades and IDH status. The novel analysis was superior to MSP testing in the validation cohort. Taken together, the novel analysis model offers an optimal clinical predictive performance of *MGMT* pyrosequencing results, and it is suitable for clinical practice in gliomas.

NEURO-COGNITIVE OUTCOMES

NCOG-01. PRESERVATION OF NEUROCOGNITIVE FUNCTION (NCF) WITH HIPPOCAMPAL AVOIDANCE DURING WHOLE-BRAIN RADIOTHERAPY (WBRT) FOR BRAIN METASTASES: PRELIMINARY RESULTS OF PHASE III TRIAL NRG ONCOLOGY CC001

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PURPOSE: NRG CC001, a phase III trial of WBRT plus memantine with or without hippocampal avoidance, sought to evaluate the neuro-protective effects of avoiding the hippocampus using intensity-modulated radiotherapy. METHODS: Adult patients with brain metastases were stratified by RPA class and receipt of prior radiosurgery/surgery and randomized to WBRT+memantine (WBRT+M) versus hippocampal-avoidant WBRT+memantine (HA-WBRT+M) (30Gy in 10 fractions). Standardized NCF tests were performed at baseline, 2, 4, 6, and 12 months. The primary endpoint was time to NCF failure, defined as decline on at least one of the following tests using the reliable change index: Hopkins Verbal Learning Test-Revised, Trail Making Test, or Controlled Oral Word Association. Cumulative incidence was used to estimate time to NCF failure (death without NCF failure was treated as competing risk) with between-arms differences tested using Grays test. To detect an 11% absolute reduction in 6-month NCF failure, 382 analyzable patients were required for 90% power with two-sided =0.05. Due to possible non-compliance, the sample size was increased by 25% (510 patients). RESULTS: From July 2016 to March 2018, 518 patients were randomized. Median age was 61.5 years. Median follow-up for alive patients was 6.1 months. Treatment arms did not differ in grade3 toxicity, overall survival, intracranial progression, or baseline NCF. Time to NCF failure was significantly longer in favor of HA-WBRT+M (p=0.012). The 6-month NCF failure rates were 69.1% (95% CI:61.8–75.3%) vs. 58.0% (95% CI:50.2–64.9%) for WBRT+M vs. HA-WBRT+M, respectively. After adjusting for stratification factors, HA-WBRT+M (hazard ratio (HR)=0.73, 95%CI:0.56–0.94, p=0.016) and age 61 years (HR=0.61, 95%CI:0.46–0.81, p=0.0006) remained significant. CONCLUSION: Preliminary analysis confirms that conformal avoidance of the neuro-regenerative hippocampal stem cell compartment during WBRT preserves neurocognitive function while achieving similar intracranial control and survival. Supported by grants UG1CA189867 (NCORP), U10CA180868 (NRG Oncology Operations), DCP from the National Cancer Institute.

NCOG-02. LONGITUDINAL AND PROSPECTIVE NEUROBEHAVIORAL OUTCOMES IN NEWLY-DIAGNOSED PRIMARY CNS LYMPHOMA PATIENTS TREATED WITH PRIMARY CRANIAL RADIOTHERAPY COMBINED WITH OR WITHOUT MTX-BASED CHEMOTHERAPY

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BACKGROUND: Primary central nervous system lymphoma (PCNSL) is an uncommon disease. Conventional treatment has consisted of either whole-brain radiotherapy (WBRT) or methotrexate (MTX)-based combined modality therapy combining chemotherapy and cranial irradiation. The addition of chemotherapy to cranial RT has significantly improved survival outcomes. However, delayed treatment-related cognitive sequelae have emerged as a significant debilitating complication of combined modality treatment in PCNSL patients, especially when effective treatment can result in disease control and greater survival. Furthermore, the specific contribution of the disease *per se* and various treatment modalities to cognitive impairment remains unclear because it is difficult to differentiate the individual neurotoxic effects of combined modalities when each can lead to cognitive dysfunctions respectively. Methods: A prospective observational cohort study with longitudinal assessments of neurobehavioral functions, neuroimaging, and activities of daily living in newly-diagnosed PCNSL patients was undertaken at our institute. Neurobehavioral outcomes were integrated into this prospective study and a battery of neuropsychological measures was used to evaluate neurocognitive functions (NCFs). The battery is composed of ten standardized NCF tests, representing four domains sensitive to disease and treatment effects (executive function, attention, verbal memory, psychomotor speed), and activities of daily living. RESULTS: Totally 15 patients with newly-diagnosed PCNSL including two cases with primary intraocular lymphoma were consecutively enrolled from February 2014 to January 2018. Comparing the differences in NCF scores between the baseline and post-treatment intervals, neurobehavioral outcomes consistently remained improving or in almost every domain evaluated in this study. Specifically, the scores of executive functions based on Paced Auditory Serial Addition Test (PACT) significantly improved between the baseline and post-chemoradiation assessment (Wilcoxon rank sum test, $p = 0.016$). CONCLUSIONS: Under the multidisciplinary treatment guidelines implemented at our institute, both improving neurobehavioral outcomes and maintaining oncological outcomes can be achieved.

NCOG-03. INDICATION OF AWAKE SURGERY FOR THE PATIENTS WITH GLIOBLASTOMA FROM THE VIEW POINT OF FUNCTIONAL INDEPENDENCE IN THE CHRONIC PHASE

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BACKGROUND: A growing number of studies demonstrated that awake surgery for lower-grade glioma results in both good functional and oncological outcome. However, indication of awake surgery for glioblastoma (GBM) has not been defined. Here, we intend to determine the indication of awake surgery for GBM based on the functional data at chronic phase. METHODS: A total of 29 patients with GBM who underwent awake surgery between May 2012 and March 2018 were included (age: mean, 52.7; standard deviation [SD], 11.5). Additionally, 41 GBM patients (age, 65.4; SD, 11.4) who underwent surgery with general anesthesia (GA) were included as historical control. The Karnofsky Performance Status (KPS) of both groups were collected at pre- and postoperative 3 month (chronic phase). Moreover, to investigate factors relating to KPS score at chronic phase, multivariate analysis with following explanatory variables were performed: age, preoperative KPS score, genetic mutation, eloquent area, resected volume, laterality, and time of surgery. RESULTS: The rate of KPS score preservation was significantly higher in awake surgery group (72.4%) than that of GA group (51.2%, p=0.03). Factors that influence the KPS score at chronic phase were age and preoperative KPS score (p=0.014, p. CONCLUSIONS: Awake surgery for GBM patients is useful to preserve independence level at chronic phase. In the view of preservation of preoperative independence level, indication of awake surgery is KPS ≥ 90 and age ≤ 64.

NCOG-04. EFFECTS OF PROTON RADIATION ON BRAIN STRUCTURE AND FUNCTION IN LOW GRADE GLIOMA

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INTRODUCTION: Previous studies have shown progressive global brain volume loss over a 1–2 year period after treatment for glioblastoma with conventional chemoradiation. The pathogenesis of this toxicity is not well understood, though may reflect unintentional exposure of normal brain to photon irradiation. Proton, as compared to photon, irradiation reduces exposure to brain areas outside the target. To explore the possibility that proton radiation therapy limits neurotoxicity seen in our previous studies, we followed a cohort of patients treated with proton radiation over a 2-year period with serial imaging and neuropsychological evaluation, in whom we have previously reported stable cognitive function. METHODS: 20 patients with low grade glioma (13 male, mean age 37.5y) were treated with proton radiation (54Gy(RBE), 1.8Gy/fx). Volumetric MRI analysis was conducted at baseline and 2 years post-treatment (interval mean/SD=26.4/4 months) on 18 subjects using an automated deep learning algorithm to segment grey and white matter in the hemisphere opposite to brain tumor. Neuropsychological evaluations were conducted on 16/18 subjects at similar timepoints (interval mean/SD=25.7/3.2 mos; 2 subjects did not complete post-treatment evaluations). RESULTS: There was no significant change in grey (t=0.98; p=ns) or white matter t=-1.46; p=ns) volume over the 2-year post-treatment interval. Neuropsychological index scores were chosen to evaluate broad domains that might be sensitive to diffuse volume loss (IQ, processing speed, working memory, delayed recall). There were no significant changes in cognitive performance. Correlations between volumetric and cognitive change were nonsignificant across all domains. CONCLUSIONS: In contrast to our prior studies demonstrating neurotoxic effects on brain volume following standard chemotherapy in combination with photon irradiation, no similar effects were observed following proton irradiation. While tumor biology effects on neurotoxicity and methodological differences are considered, proton irradiation appears to preserve brain structure and function over a comparable 2-year period. HS and JD contributed equally.

NCOG-05. INTRA-INDIVIDUAL NEUROCOGNITIVE VARIABILITY IN PATIENTS WITH CANCER: A PREDICTOR AND MARKER OF NEUROTOXICITY ASSOCIATED WITH CHEMOTHERAPY?

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There is a growing concern about neurotoxicity involving decreased neurocognitive function (NCF) associated with systemic chemotherapy. Intra-individual variability (IIV) is an index of NCF performance spread within an individual considered to be an indicator of neurocognitive control, and has been shown to worsen on reaction time tasks after chemotherapy in breast cancer. The aim of the current study was to investigate IIV as indexed by dispersion of performances across a battery of NCF tests to determine whether (1) IIV prior to chemotherapy predicts declines in NCF status and (2) whether IIV increases during and shortly after chemotherapy. METHODS: Forty-two women with breast cancer were administered a standardized neuropsychological battery that included tests of learning and memory, attention, processing speed, and executive function prior to and during/shortly after chemotherapy. To compute covariance of variation (CoV), intra-individual standard deviation across normatively-adjusted z-scores was generated and divided by the mean of these scores. Patients were assessed for depression using the BDI-2. RESULTS: Baseline IIV was higher in patients that declined (mean[SD]= 3.19[3.94]) compared to patients that were stable or improved after chemotherapy (1.43[0.88]) with a medium to large effect size (Hedge's g=.62, p=.12). Paired samples t-test revealed that IIV did not significantly change on average from before to during/after chemotherapy (Hedge's g=.18, p=.42). Trends were observed for a correlation between worsened IIV over time and worsened mean-level neuropsychological performance (r =-.29, p=.08), but not with changes in depression (r =.03, p=.87). CONCLUSIONS: Neurocognitive IIV prior to chemotherapy was associated with declines in cognitive performance, although changes in IIV after chemotherapy were not evident. Higher levels of neurocognitive dispersion prior to treatment may serve as an important risk factor for susceptibility to neurotoxic effects of systemic chemotherapies. Confirmation of these results in larger samples and diverse cancer and treatment types are warranted.

NCOG-06. PREDICTING TUMOR TREATING FIELD COMPLIANCE USING NEUROCOGNITIVE TESTING

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PURPOSE: To determine if neurocognitive functional status can be used as a predictor of Tumor Treating Field (TTFields) use and compliance METHODS AND MATERIALS: Twelve patients diagnosed with primary GBM underwent a battery of neurocognitive tests to determine their neurocognitive functional status. A retrospective analysis was performed to determine if neurocognitive function is related to TTField use and compliance. RESULTS: Of the 12 patients, 10 were male and the median age was 64 years. ECOG performance status was 0 or 1 in all patients. Nine initiated TTFields therapy, and we followed their TTField use and compliance. We reviewed the mean score on each of the neurocognitive tests in TTField users and non-users and found no significant difference. We also analyzed the means of the TTField users and compared those who had a compliance of > 75% versus 0–75%. In this comparison, we found that patients who had a compliance of > 75% trended towards better neurocognitive function in 2 of the tests, the Trailmaking Test A (TMTA) and the Test of Premorbid Function (TOPF). The TMTA is a measure of simple visual-motor processing speed and visual attention, and the TOPF represents an estimation of the patients premorbid intellectual abilities. The mean TMTA z-scores were -3.40 in patients who had a compliance of > 75% and -0.943 those with compliance of 0–75% (p=0.080). The mean TOPF standard scores were 108 in patients with compliance > 75% compared to 88.7 in those with a compliance of 0–75% (p=0.062). CONCLUSIONS: Neurocognitive testing may be used a potential predictor of TTField compliance. In our small patient population, those with higher premorbid intelligence and faster psychomotor speed tended to have better compliance in TTField use. We expect that further investigation, in a larger dataset, may lead to a more refined neurocognitive profile of TTField compliance.

NCOG-07. NEUROCOGNITIVE EVALUATION IN ELDERLY MALIGNANT GLIOMA PATIENTS

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BACKGROUND: Glioblastoma (GBM) is the most common primary brain tumor in adults, with an increasing incidence in patients aged 75

through 85. Prognosis in these patients is particularly dismal due to more aggressive tumor biology, lower functional reserve and high prevalence of comorbidities. Many studies report cognitive impairment in GBM patients, ranging from 29 to 90%. This study was aimed at evaluating neurocognitive status and comorbidities of an elderly population with high grade glioma and the correlation with clinical and demographical variables. METHODS: Patients underwent an extended neuropsychological evaluation with a battery of standardized tests on 8 cognitive domains: global function (GF); verbal learning (VL); short and long-term memory (STM); executive functions (EF); abstract reasoning (AR); attention (ATT) and visuo-constructional abilities (CA). Moreover, the Cumulative Illness Rating Scale was administered to each patient for comorbidities evaluation. RESULTS: We assessed 69 patients with median age at diagnosis of 74 years (range 65–85). 43 patients (62%) presented multi-domain cognitive impairment, and only 8 (12%) showed no cognitive impairment. Neuropsychological deficit mainly affected executive functions (n=42), short term memory (n=28), long term memory and attention (n=22). Patients with AR deficit had a poorer PFS and OS (p<0.001%). At the follow up, 7 out of 12 patients showed cognitive improvement, 4 resulted furtherly deteriorated and 1 patient was stable. Attention was the most affected function at follow up, while verbal learning was the most improved one. CONCLUSIONS: Our results highlight the high prevalence of cognitive deficits in patients with Glioma. Moreover, this study underlines the need to include cognitive functioning and comorbidities evaluation in the assessment of elderly neuro-oncological patients.

NCOG-08. DETERMINING THE CONTENT VALIDITY OF MEASURES OF BASIC AND INSTRUMENTAL ACTIVITIES OF DAILY LIVING (ADL) IN PATIENTS WITH BRAIN TUMORS: A SYSTEMATIC REVIEW

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BACKGROUND: Brain tumor patients may experience impairments in everyday functioning due to both physical and cognitive symptoms. Measures of everyday functioning may therefore be relevant for brain tumor patients in both clinical trials and practice. Everyday functioning can be assessed using activity of daily living (ADL) outcome measures. There are two categories of ADL: basic activities of daily living (BADL; e.g. washing and dressing) and instrumental activities of daily living (IADL; e.g. shopping or cooking). The aim of this systematic review was to identify outcome measures containing items on BADL and/or IADL that currently are used in studies with brain tumor patients, and to assess the content validity of these instruments. METHODS: Several electronic databases (i.e. PubMed, Embase, Cochrane, PsycINFO and CINAHL) were searched up to April 2017 to identify studies with brain tumor patients that used outcome measures with ADL items. Articles were selected based on predetermined in- and exclusion criteria. RESULTS: The literature search identified 31 unique outcome measures containing items on BADL and/or IADL. There were 22 (71%) outcome measures containing BADL items and 29 (94%) outcome measures containing IADL items. More than half (65%) of the outcome measures contained both BADL and IADL items. The number of BADL items in each questionnaire ranged from 0% to 83%, and from 0% to 100% for IADL items. Only two outcome measures were specifically developed to measure BADL (Barthel Index and Katz-ADL), and two specifically for IADL (Lawton-Brody IADL and preliminary IADL-brain tumor). However, these instruments have not (yet) been validated in brain tumor patients. CONCLUSION: Currently, there is a lack of suitable options to sufficiently measure BADL and/or IADL in brain tumor patients. Validation of currently existing BADL and IADL measures in brain tumor patients seems necessary, or the development of new tools.

NCOG-09. THE LEVEL OF REPORTING OF NEUROCOGNITIVE OUTCOMES IN RANDOMIZED CONTROLLED TRIALS OF BRAIN TUMOR PATIENTS: A SYSTEMATIC REVIEW

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INTRODUCTION: Neurocognitive impairment is frequently present in brain tumor patients and is therefore considered an important outcome in brain tumor research. To use neurocognitive outcomes (NCO) in clinical decision-making, neurocognitive evidence should be of sufficiently high quality. One important aspect in the generation of high quality evidence is the level of reporting. We aimed to investigate the level of neurocognitive functioning reporting in randomized controlled trials (RCTs) in brain tumor patients. METHODS: We conducted a systematic literature search in several databases up to August 2017. Of the selected relevant RCTs, the following data were retrieved: basic trial demographics and NCO characteristics, quality of NCO reporting, and risk of bias. We also analyzed studies that should impact clinical decision-making based on their quality of reporting. RESULTS: We identified 65 RCTs, of which NCO was the primary endpoint in 14 (22%). The included brain tumour types varied, with 20 RCTs studying glioma patients (31%) and 17 studying brain metastatic patients (26%) only. Radiotherapy and chemotherapy were the most studied treatments (both in 31% of studies). In five studies (8%), rehabilitation of neurocognitive impairments was investigated. Important methodological limitations were related to the documentation of statistical approaches for dealing with missing data, and to discussing limitations and generalizability issues uniquely related to the NCO components. Risk of bias was high regarding blinding of personnel and incomplete outcome data. Twenty RCTs (31%), eight with NCO as primary endpoint and 12 as secondary endpoint, satisfied a sufficient number of criteria to be classified as high-quality NCO evidence. Most of these studies did contribute to clinical decision-making. CONCLUSION: Investigators involved in brain tumor research should give attention to methodological challenges related to NCO reporting as identified in this review, as high-quality reporting of NCO evidence can be of value in clinical decision-making.

NCOG-10. EXECUTIVE DYSFUNCTION IN NEUROONCOLOGY: BEHAVIOR RATING INVENTORY OF EXECUTIVE FUNCTION IN ADULT PRIMARY BRAIN TUMOR PATIENTS

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BACKGROUND: Adult primary brain tumor (PBT) survivors report persistent cognitive difficulties throughout treatment which are problematic for everyday functioning, employment capability, and quality of life. Cognitive domains often affected by cancer treatment appear to be attention and executive functioning (EF). One validated measure developed to assess an individual's EF within daily living is the Behavior Rating Inventory of Executive Function – Adult (BRIEF-A). To date, no published research has investigated the EF profile of PBT patients using the BRIEF-A. METHODS: Seventy-four patients from a NCI-designated cancer center completed the BRIEF-A, a validated self-report questionnaire that assesses executive functioning across nine subscales and provides three index scores: Behavioral Regulation, Metacognition, and Global Functioning. Descriptive analyses were conducted to determine the self-reported EF profile in PBT patients. MANOVA's compared the performance of PBT patients to three diagnostic populations (comparison group data obtained from BRIEF-A manual): mild cognitive impairment (MCI), unmedicated attention-deficit/hyperactivity disorder (ADHD-U) and healthy controls (HC). RESULTS: PBT BRIEF-A group means were average across subscales and indexes, yet the prevalence of significant elevations ranged from 12–50%. The Metacognition Index demonstrated a 38% elevation prevalence compared to 22% in Behavioral Regulation. Approximately 61% of the sample had at least one clinically elevated scaled score. When comparing between group profiles, PBT reported significantly more impairment than HC and significantly less than ADHD-U across all subscales. No significant differences were found between PBT and MCI groups. CONCLUSIONS: Despite group means not reaching clinical impairment, a substantial proportion of patients with PBT endorse significant difficulty with executive dysfunction. Elevations were most prominent in metacognitive abilities (e.g., Working Memory), over behavioral dysregulation (e.g., Emotional Control). Notably, the EF profile of PBT patients was remarkably similar to that of MCI, increased when compared to HC, and well below ADHD-U.

NCOG-11. FEASIBILITY AND EFFICACY OF AN IPAD-BASED COGNITIVE REHABILITATION PROGRAM IN BRAIN TUMOR PATIENTS

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OBJECTIVE: To assess feasibility and effect on cognitive function and Health-Related Quality of Life (HRQoL) of an iPad-based intervention in grade 2 and 3 glioma patients stable off treatment. Patients with lower grade glioma suffer significant cognitive dysfunctions that impact their HRQoL. Formal cognitive rehabilitation is a limited resource that may be more available if deployed with a mobile device such as an iPad. METHODS: Stable,

grade 2 and 3 glioma patients with subjective cognitive complaints, complete a baseline computerized battery of standardized cognitive tests using the NIH Toolbox and HRQOL assessment with the FACT-BR. Patients then completed a novel, evidence-based, iPad based, brain tumor specific, cognitive rehabilitation program called ReMind over the next 3 months (~3 hours per week). NIH Toolbox and HRQOL assessments were repeated after completion of the rehabilitation, and again 9 months after baseline. Primary endpoint was feasibility with secondary endpoints of changes in cognitive scores and HRQOL assessments. RESULTS: To date, 10 patients have enrolled and completed baseline testing, of whom 5 have completed ReMind rehabilitation. Median age is 56 years. Median disease duration is 7.6 years. 5 patients have Oligodendrogliomas (IDH mutated and 1p19q deleted), 3 patients have Astrocytomas, IDH mutated, and 2 patients have Astrocytomas NOS. 5 are grade II and 5 are grade III. 5 had left hemisphere tumors, 4 had right hemisphere tumors, and 1 was bilateral. 10 had prior chemotherapy and 8 prior radiation. We anticipate enrolling another 5 – 10 patients and will present the updated feasibility data as well as changes in cognitive and HRQOL scores. CONCLUSION: As patients with lower grade tumors live longer, it is important to increase availability of cognitive interventions to improve HRQOL and outcomes. This iPad based approach provides in-home access to cognitive training and compensation strategies for patients with brain tumors.

NCOG-12. COMPREHENSIVE GERIATRIC ASSESSMENT (CGA) FOR OUTCOME PREDICTION IN ELDERLY PATIENTS (PTS)WITH GLIOBLASTOMA (GBM): A MONO-INSTITUTIONAL EXPERIENCE

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BACKGROUND: Treatment for GBM elderly PTS is still a challenge in neuro-oncology. Clinical tools, including CGA, are needed for improving treatment decision and outcome. The aim of this study was to evaluate CGA as a prognostic tool in terms of PFS and OS in elderly GBM PTS. METHODS: We performed a retrospective analysis of elderly PTS ≥ 65 years, treated at Veneto Institute of Oncology between January 2011 and January 2018, with newly histologically diagnosed GBM and receiving a baseline CGA after 3–4 weeks from surgery. CGA included the following domains: age, activities and instrumental activities of daily living (ADL, IADL), cognitive status (MMSE), mood (GDS), nutritional status (MNA), number of drugs, comorbidity (cumulative Illness Rating Scale-CIRS), presence of geriatric syndromes, presence of caregiver. PTS were classified according to Balducci's criteria into Fit or Unfit (Frail and Vulnerable). RESULTS: 113 PTS were enrolled: 72(64%) were male, KPS were ≥ 70 in 90 PTS(80%); 37 PTS(33%) had a radical surgery, 63% partial surgery and 4% received a biopsy. 90 PTS(80%) received Stupp treatment, 16 (14%) temozolomide or radiotherapy alone and, only 7(6%) received no treatment. MGMT methylation status was analyzed in 96 PTS: 44% were metMGMT. According to CGA evaluation: 40 PTS(35.4%) were classified as Fit and 73 PTS(64.6%) Unfit. PFS was 11.2(95% CI 6.0–16.4) and 7.2(95% CI 5.8–8.6) months for Fit and Unfit PTS (p=0.1). On multivariate analysis, adjusted for type of surgery, MGMT methylation status and type of therapy, PFS was significantly different between the two groups (HR=0.6, 95% CI 0.2–0.9; p=0.04). OS was 16.4(95% CI 14.6–18.2) and 10.6(95% CI 8.3–12.8) ms for Fit and Unfit PTS (p=0.04); on multivariate analysis the HR was 0.51(95% CI 0.2–0.9; p=0.04). CONCLUSIONS: CGA demonstrated significant outcome prediction in terms of OS and PFS, regardless of therapy and it could be a useful treatment decision-tool.

NCOG-13. NEUROCOGNITIVE EVALUATION OF BRAIN METASTASES PATIENTS TREATED WITH POST-RESECTION STEREOTACTIC RADIOSURGERY: A PROSPECTIVE SINGLE ARM CLINICAL TRIAL

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OBJECTIVE: Post-operative radiation therapy for brain metastases (BM) has become standard treatment. Concerns regarding the deleterious cognitive effects of Whole Brain Radiation Therapy (WBRT) spurred a trend to use focal therapies such as stereotactic radiosurgery (SRS). The purpose of this study was to prospectively evaluate the neuropsychological fluctuations that follow post-resection SRS treatment since limited data exist in this context. METHODS: We conducted a prospective single arm cohort study of patients with 1–2 BM, who underwent resection of a single BM between May 2015 to December 2016. Patients were evaluated for cognitive functions (NeuroTrax test) and quality of life (QOL; QLQ-30, QLQ-BN20) before and 3 months following post-resection SRS. Results: Twelve out of 14 patients completed pre- and post SRS neurocognitive assessments. Overall, we did not detect significant neurocognitive or QOL changes 3 months following SRS. In a subgroup analysis, cognitive changes among patients at the age of 60 or over (n=7) were compared to younger ones (n=5). Both age groups were similar in terms of gender, pre-treatment KPS and ECOG, total SRS treatment volume, lesion eloquence and post-SRS survival. Among patients younger than 60 years, median global cognitive score increased from a pre-treatment score of 88 (72–102) to 95 (79–102), 3 months following SRS treatment, p=0.019; Wilcoxon paired non-parametric test. Immediate verbal memory and executive functions scores increased from 86 (72–98) to 98 (92–112) and 86 (60–101) to 95 (73–108), respectively, p=0.043. No significant cognitive changes were discovered among patients at the age of 60 or older. Conclusion: Post-resection radiosurgery has a safe neuro-cognitive profile and is associated with a relatively preserved quality of life. Patients younger than 60 years benefit more than older ones and may even regain some cognitive functions within a few months after treatment.

NCOG-14. HIPPOCAMPAL N-ACETYLASPARTATE CONCENTRATION CORRELATES TO VERBAL MEMORY BEFORE RADIOTHERAPY FOR BRAIN METASTASES

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BACKGROUND: Changes of quality of life and cognitive function, especially verbal memory, after brain radiotherapy is currently widely discussed in neurooncology with substantial change in the paradigm of treatment of brain metastases. In our previous study, we described the decrease in the hippocampal concentration of N-acetylaspartate (NAA, marker of neuronal density and viability) in response to whole brain radiotherapy (WBRT). The aim of presented analysis is to evaluate the NAA concentration before radiotherapy and to describe the relation to the verbal memory baseline. MATERIAL AND METHODS: Patients with brain metastases indicated to WBRT underwent hippocampal MR spectroscopy (MRS) along with neuropsychological examinations focused among others to verbal memory. Absolute NAA concentrations for right and left hippocampus and the sum of absolute NAA concentrations in both hippocampi were compared with results of AVLT_TR. RESULTS: The examination was performed in 26 patients. Patients were divided into two groups based on median NAA concentration (8.56 mM). The median AVLT_TR was 37 points in the group with hippocampal NAA concentration < 8.56 mM. In the group of patients with baseline NAA concentration ≤ 8.56 mM, the median AVLT_TR was higher with 43 points (Mann-Whitney U Test, p = 0.02). CONCLUSION: Non-invasive examination by hippocampal MRS can predict the baseline pre-radiotherapy cognitive functions, which are normally tested by time-consuming psychological tests. Patients who had lower baseline NAA hippocampal concentrations had a significantly lower baseline verbal memory. NAA hippocampal concentrations may be a useful biomarker for selecting patients who would benefit most from the hippocampal sparing radiotherapy techniques reducing the risk of iatrogenic deterioration of QoL in patients treated with a palliative intent, especially in cases where local brain stereotactic radiotherapy is not applicable or available.Supported by the Ministry of Health of the Czech Republic, grants NV18-03-00469, NV18-03-00398.

NEURO-IMAGING

NIMG-01. A BLINDED IMAGE EVALUATION STUDY TO DETERMINE THE DIAGNOSTIC EFFICACY OF ^{18}F-FLUCICLOVINE PET, AS AN ADJUNCT TO MRI IMAGING, IN ADULTS WITH GLIOMA

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The Response Assessment in Neuro-Oncology (RANO) working group have emphasized the clinical utility of Positron Emission Tomography (PET) imaging in brain tumour, highlighting the superiority of amino acid PET tracers over glucose PET tracers in diagnosis, treatment monitoring, and response assessment (Albert, 2016). The amino acid PET tracer ^{18}F-fluciclo-

vine is commercially available for prostate cancer imaging in the US and EU and has Orphan Drug designation for imaging glioma in both jurisdictions. This blinded image evaluation determined the diagnostic performance of ^{18}F-fluciclovine PET (as an adjunct to contrast-enhanced T1-weighted (CE-T_1W) magnetic resonance imaging (MRI)), when interpreted by experienced PET-CT readers naïve to ^{18}F-fluciclovine. Thirty-five PET and MRI (CE-T_1W and fluid-attenuated inversion recovery (FLAIR)) image datasets, with corresponding histological standard-of-truth, collected during a prospective clinical trial were evaluated. A neuroradiologist read the MRI images and three nuclear medicine physicians, blinded to any patient-specific medical information, independently read each ^{18}F-fluciclovine image (alongside the CE-T_1W MRI). Positive/negative predictive values, sensitivity and specificity were determined for CE-T_1W, FLAIR and for ^{18}F-fluciclovine plus CE-T_1W. Inter- and intra-reader reproducibility was assessed by determining agreement in diagnostic performance parameters and reader-defined Volumes of Interest using intersections and similarity metrics. Date of completion of planned analysis: 22nd June 2018. References: Albert N et al. Response Assessment in Neuro-Oncology working group and European Association for Neuro-Oncology recommendations for the clinical use of PET imaging in gliomas. Neuro-Oncology 18(9), 1199–1208, 2016

NIMG-02. STRAIN ELASTOGRAPHY: INTRA-OPERATIVE BRAIN TUMOUR CARACHTERIZATION

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BACKGROUND: Sonoelastography is an ultrasound imaging technique able to assess mechanical properties of tissues. Strain elastography (SE) is qualitative sonoelastographic modality with a wide range of clinical applications, but its use in brain tumor surgery is very limited. OBJECTIVE: We describe the first large-scale implementation of SE in oncological neurosurgery for lesions discrimination and characterization. METHODS: We analyzed retrospective data from 64 patients aiming at (i) evaluating the stiffness of the lesion and of the surrounding brain, (ii) assessing the correspondence between B- mode and SE, and (iii) performing subgroup analysis for gliomas characterization RESULTS: i) In all cases we visualized the lesion and the surrounding brain with SE, permitting a qualitative stiffness assessment. ii) In 90% of cases, lesion representations in B-mode and SE were superimposable with identical morphology and margins. In 64% of cases lesion margins were sharper in SE than in B-mode. iii) In 76% of cases, glioma margins were sharper in SE than in B-mode. Lesions morphology/dimensions in SE and in B-mode were superimposable in 89%. Low grade (LGG) and high grade (HGG) gliomas were significantly different in terms of lesion stiffness, stiffness of the surrounding brain, and stiffness contrast between tumours and brain, LGG appearing stiffer while HGG softer than brain (all p CONCLUSION: SE allows to understand mechanical properties of the brain and lesions in exam and permits a better discrimination between different tissues compared to B-mode. Additionally, SE can differentiate between LGG and HGG.

NIMG-03. RADIOMIC TEXTURE ANALYSIS TO PREDICT RESPONSE TO IMMUNOTHERAPY

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BACKGROUND: Radiomic texture analysis (TA) from standard MRI imaging may be able to discriminate between responders versus non-responders in glioblastoma patients treated with pembrolizumab immunotherapy. METHODS: 14 patients (5 males; mean age 58 years; range: 32–72 years), with pathologically-proven recurrent GBM, enrolled in a pembrolizumab clinical trial, were retrospectively evaluated. Immunotherapy Response Assessment in Neuro-Oncology(iRANO) were performed. Patients were categorized based on: 1) best response or 2) overall response (OR) using the iRANO status at the last scan time in the trial. Patients with progressive disease (PD) were classified as non-responders, while patients with partial response (PR) or stable disease (SD) were classified as responders. T2-FLAIR (edema/invasion) and post-contrast T1WI (enhancing tumor) of baseline scans were co-registered and segmented (3D Slicer, v.4.3.1) to create a volume of interest for Radiomic TA. A total of 4880 texture features were extracted. Feature selection was performed using Lasso regularization. For classification and predictive model building, gbtree booster of XGboost with Leave-One-Out Cross-Validation (LOOCV) was used on the selected texture features to build a binary logistic regression model and classify the patients into respective groups<.strong> RESULTS: Using the best response classification, 10 patients were classified as non-responders and four patients classified as responders (1 SD; 3 PR). Using 13 radiomic features, these patients could be classified into their respective responding groups with a sensitivity, specificity and accuracy of 100%, p-value=0.0089. Based on OR, 12 patients were classified as non-responders and two as responders (2 SD). Seven features were able to differentiate the responding patients with a sensitivity, specificity and accuracy of 100%, p-value=0.0089. CONCLUSION: Radiomic TA was able to discriminate and predict those GBM patients that are responders versus non-responders to pembrolizumab with high robustness. Of note, given the small number of patients in this cohort, a larger cohort of patients is needed to minimize overfitting.

NIMG-04. DIFFUSION RESTRICTION ON MR IMAGING IN THE T2 HYPERINTENSE, BUT OTHERWISE NORMAL-APPEARING WHITE MATTER OF GLIOBLASTOMA PATIENTS TREATED WITH TTFIELDS CORRELATES WITH SURVIVAL

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INTRODUCTION: Based on the positive results of our pilot study that evaluated MR diffusion tensor imaging characteristics in the white matter of 13 subjects with glioblastoma, we aimed to evaluate the prognostic value of diffusion restriction in a new, larger cohort of glioblastoma patients. METHODS: Thirty-five subjects diagnosed with glioblastoma were treated by surgical resection, radiotherapy, temozolomide and TTFields (Optune®). All subjects were followed by MRI at 1.5 T approximately every two months (total range 160–1810 days) with standard imaging, including diffusion-weighted imaging, until tumor progression. Apparent diffusion coefficient (ADC) maps were calculated using FSL and freehand ROIs were placed on B0 images within the T2 hyperintense white matter (T2HWM) near the resection cavity by a single investigor (AMR), and then projected onto the ADC maps. Additionally, an ROI was similarly placed within the normal-appearing white matter (NAWM). Final values (ADC-ratio) were calculated by dividing ADC in T2HWM by ADC in NAWM. Linear regression slopes (ADC-ratio vs. time) for individual subjects were fitted in R. RESULTS: In 26 subjects a positive slope was observed in ADC-ratio over time, while in the remaining subjects the slope was negative. We found a significant difference between subjects with positive and negative regression slopes in overall survival (OS; hazard ratio = 0.27 [0.10.69], log rank p = 0.009) and progression-free survival (PFS; hazard ratio = 0.31 [0.120.81], log rank p = 0.02), with positive regression slope associated with more favorable outcome. CONCLUSION: Our data suggest that increasing ADC in the affected (T2 hyperintense) white matter of glioblastoma patients is favorable, with significantly greater PFS and OS in patients with increasing ADC over time. Changes in ACD likely reflect changes in tumor density in the affected white matter, with decreasing values (increasing diffusion restriction) corresponding to tumor progression.

NIMG-06. KINETICS-BASED RESPONSE METRIC DISCRIMINATE IMPROVED OUTCOMES FOR PATIENTS RECEIVING BEVACIZUMAB-BASED THERAPIES

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INTRODUCTION: Evaluating treatment response in glioblastoma is a difficult task for clinicians, particularly in recurrent disease. Current response metrics focus on changes in imageable tumor burden which can be ambiguously affected by anti-angiogenic therapy. In previous work, we reported the ability of the Days Gained (DG) response metric to discriminate patients receiving bevacizumab into long and short-term survival groups. In this work, we investigate how the DG metric performs for patients who received bevacizumab with a concurrent cytotoxic agent (e.g. CCNU) compared to patients who received bevacizumab alone. METHODS: We identified a set of 38 patients with recurrent glioblastoma who received bevacizumab therapy (21 patients) or bevacizumab with concurrent CCNU (17 patients). Each case had tumor volumes segmented on two dates prior and one day post therapy on T1GD and FLAIR MR imaging. DG scores were calculated using tumor growth characteristics and were used to evaluate discrimination of patient survival and time to progression (TTP) using Kaplan-Meier curves and logistic regression. RESULTS: An optimal threshold of 162 DG (p= 0.0393, log rank) on T1GD imaging was discriminative for TTP

in bevacizumab cases. Combination therapy with CCNU showed a similar trend for the correlation between DG and TTP outcomes as for bevacizumab alone. Patients receiving bevacizumab alone had significant DG thresholds for survival following treatment (T1GD: 162 DG, p=0.01857; FLAIR: 216 DG, p=0.0387). Although predictive of TTP, combination therapy was not significantly associated with increased overall survival from time of treatment. DISCUSSION: Days gained was able to discriminate survival and TTP for patients receiving bevacizumab therapy alone. Discriminative power for combination therapy with CCNU demonstrated the same trend for TTP outcomes. CONCLUSION: Growing evidence supports DG as a clinically meaningful metric of treatment response even in the context of anti-angiogenic therapies that are known to ambiguously modulate imaging features on MRI.

NIMG-07. DEEP LEARNING DETECTS DIFFERENCES IN THE MRIs OF MALE AND FEMALE GLIOMAS

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INTRODUCTION: While a growing body of research on the intrinsic biological differences between male and female tumors and their environments continues to evolve, few deep learning techniques have been trained with a sex-specific focus. To investigate the influence of sex differences on automated tumor segmentation, a set of Male and Female 3D deep convolutional neural networks were trained and evaluated (MaleDNN and FemaleDNN). METHODS: A balanced data set was obtained from our brain tumor database of 518 cases with known sex, pretreatment T1GD and FLAIR MRI, and at least one brain tumor segmentation. Cases were split by sex to create training cohorts of 200 male and female cases to train the MaleDNN and FemaleDNN. A set of 59 unseen test cases for each sex were reserved for evaluation. Both networks were used to segment tumor volumes from male and female test cases. Whole-tumor Dice coefficients (1=perfect overlap, 0=no overlap) were calculated by comparing network segmentations against segmentations from trained measurers. RESULTS: The MaleDNN had higher overall performance on all cases (Dice male=0.8416; female=0.7800) compared to the FemaleDNN (Dice male=0.8269, female=0.7639). The difference in performance between networks was significant for male tumors (p=0.0466), but not female tumors (p=0.1872). Both networks performed better on male tumors. Average dice scores were significantly lower for the MaleDNN (Dice decrease=0.0616, p=0.0273) and FemaleDNN (Dice decrease=0.629, p=0.0422) when evaluating female tumors. DISCUSSION: It was anticipated that the MaleDNN and FemaleDNN would have highest performance on test cases from the same sex. However, the FemaleDNN performed better on males than females and the MaleDNN had comparable performance for female tumors. The significant differences between these sex-specific deep neural networks indicate that male and female gliomas differ on imaging and that female tumors are more difficult to segment at initial presentation.

NIMG-08. REPRODUCIBILITY OF HYPERPOLARIZED C-13 METABOLIC IMAGING IN PATIENTS WITH GLIOMA

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As a non-invasive method for probing in vivo metabolism, hyperpolarized carbon-13 metabolic imaging may enhance the diagnostic assessment of response-to-treatment in patients with glioma. Having demonstrated the feasibility of applying this technique to human glioma (Park et al. 2017), the current study seeks to evaluate the reproducibility of carbon data by measuring serial metabolic changes in white matter. In total, 4 radiologically stable patients (2 male, 2 female) who were treated with radiation and TMZ for heterogeneous diagnoses (GBM, grade III oligo, gliosarcoma, secondary GBM) received either 2 (n=1) or 3 (n=3) serial exams. Following injection of hyperpolarized [1-^{13}C]pyruvate, dynamic echo-planar imaging was acquired with a 3s temporal resolution and 2–8 cm^3 spatial resolution. Dynamic data were summed together and the ratio of metabolically-produced lactate relative to pyruvate substrate (lac/pyr) was evaluated in segmented normal-appearing white matter (NAWM, >50% voxel). Median and SD of lac/pyr values in NAWM are reported for serial scans, along with %change per interval: P1-male [0.32 ± 0.14, 0.28 ± 0.11 (-12.5%), 0.33 ± 0.10 (+17.9%)]; P2-male [0.32 ± 0.10, 0.28 ± 0.10 (-12.5%)]; P3-female [0.37 ± 0.09, 0.45 ± 0.14 (+21.6%), 0.59 ± 0.19 (+31.1%)]; and P4-female [0.49 ± 0.14, 0.43 ± 0.12 (-12.2%), 0.44 ± 0.10 (2.3%)]. These results show an average absolute change of 15.7% in lac/pyr within NAWM over the entire population, with considerable overlap of the variance. Patient P3, who was treated with adjuvant agent pembrolizumab, also notably showed a serial increase in lac/pyr. When separating the population according to gender, females demonstrated a higher average lac/pyr value of 0.46, compared to 0.31 in males. In summary, our initial experience with serial hyperpolarized carbon data indicates that the range of lactate-to-pyruvate ratios is relatively consistent across patient scans, but may differ according to gender and adjuvant treatment. Future studies will evaluate changes in the T2 lesion relative to NAWM over the course of treatment.

NIMG-09. iPROMPT: INTEGRATED PREDICTIVE RADIOGRAPHIC BIOMARKERS OF PROGRESSED HIGH GRADE ASTROCYTIC TUMORS

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In 2018 a projected 79,000 persons in the US will be diagnosed with a primary brain tumor. Glioblastoma (GBM) is the deadliest and most common of these tumors in adults with overall survival ~15 mos and >93% of patients deceased by 5-years. Disease progression is inevitable, and effective second-line therapies are few. Delays in identifying progressed GBM risks neurologic decline and increased tumor mutations that potentially renders treatment less-effective. Reliable brain tumor imaging can be ambiguous to distinguish true progression from treatment effect. Furthermore, inaccurate identification of tumor progression might result in continuation of ineffective treatment or premature discontinuation of effective therapies. This is an active topic in neuro-oncology as it applies to patient care and standardization of imaging techniques. Improved tumor imaging surveillance tools that supplement current standards and allow earlier detection of progression can improve clinician confidence and patient outcomes. This study collected 468 WHO grade IV astrocytomas with gross-total-resection within our institution between 2006 and 2017 to evaluate changes in T2/FLAIR signal intensity, diffusion restriction, and contrast enhancement both in and around the resection cavity to determine earliest indicators of tumor progression. Early data release of this ongoing retrospective study reveals encouraging findings that support a novel combination of radiographic biomarkers associated with GBM progression using standard brain tumor imaging. A feasibility study including 47 cases, thus far, support previously reported signal intensity changes within the resection cavity as well as specific phenomena that have not been previously described, such as *FLAIR migration* and *signal coring*. The proposed technique does not require advanced brain tumor imaging and can therefore be broadly employed and standardized in clinical practice. With further validation, this novel combination of imaging features that might serve to supplement existing response criteria.

NIMG-11. DIFFERENTIATING TREATMENT-INDUCED EFFECTS FROM TRUE RECURRENT HIGH GRADE GLIOMA USING MULTIPARAMETRIC MRI TECHNIQUES

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Recurrent high grade gliomas (rHGG) are difficult to diagnose accurately as they are often confounded with treatment-induced effects (TxE) because on conventional T1w and T2w anatomical MR imaging these two phenomena appear identical. This could result in removing a patient from an effective second-line therapy, confound the results of a clinical trial of a new therapeutic, and expose a patient to unnecessary surgical intervention. Advanced MR imaging modalities have shown promise in distinguishing between TxE and rHGG, but prior studies are unable to account for heterogeneity within the same lesion and often lack pathological confirmation. The goal of this study was to identify imaging parameters that could spatially map directly to pathology to account for heterogeneity within lesions. 1–8 tissue samples were collected upon surgical resection of suspected rHGG. A total of 484 samples were collected from 183 patients; 332 of these (163

patients) were labeled as either pathologically confirmed treatment effect or having no tumor cells (tumor score=0) within the imaging abnormality (TxE; 80/51 samples/patients) or rHGG (tumor score=2–3; 252/118 samples/patients) by a pathologist. Preoperative 3T MRI scans included T1w-Gd and T2w FLAIR; diffusion tensor imaging (b=1000s/mm2); 3D MRSI; and dynamic susceptibility-contrast perfusion MRI. Univariate models were created using generalized estimating equations to evaluate each variable's ability to distinguish TxE from rHGG tissue samples. Normalized choline (median=1.1 TxE & 1.2 rHGG), choline-to-NAA index (CNI, median=2.4 TxE & 3.5 rHGG), and normalized cerebral blood volume (nCBV, median=1.2 TxE & 1.4 rHGG) were significantly associated with biopsy-level classification (p=0.034, 0.015, 0.017). These results are being consolidated into a multiparametric algorithm for predicting TxE or rHGG, which could be of clinical importance in managing patients with recurrent rHGG. Downstream analysis will create a spatial map of rHGG to help guide future surgical sampling.

NIMG-12. RADIOGENOMICS ON VENUS AND MARS: IMPACT OF SEX-DIFFERENCES ON MRI AND GENETIC CORRELATIONS IN GLIOBLASTOMA

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BACKGROUND: MRI-based modeling can help characterize the intratumoral genetic heterogeneity of Glioblastoma (GBM). Yet, published models to date have neglected the potential impact of sex-differences on the accuracy of MRI-genetic correlations. Specifically, there is growing awareness that female GBM patients can display different genetic/molecular aberrations and phenotypic expression compared to male counterparts. In this exploratory study, we compare MRI signal and key GBM driver alterations across a cohort of male and female GBM patients, using image-guided biopsies and spatially matched multi-parametric MRI. METHODS: We collected 61 image-guided biopsies from 18 primary GBM patients (9/9 male/female). For each biopsy, we analyzed DNA copy number variants (CNV) for 6 core GBM driver genes reported by TCGA: amplifications (++) for EGFR and PDGFRA and deletions (--) for PTEN, CDKN2A, RB1, TP53. We compared regional CNV status with spatially matched MRI texture measurements from co-registered biopsy locations. Advanced MRI features included relative cerebral blood volume (rCBV) on DSC-perfusion, mean diffusivity (MD) and fractional anisotropy (FA) on diffusion tensor imaging. We identified univariate correlations for combined and sex-specific (male, female) subgroups. We also built multivariate predictive decision-tree models for each GBM driver gene and used leave-one-out-cross-validation (LOOCV) to determine area-under-curve (AUC) on ROC analysis to compare accuracies across combined and sex-specific models. RESULTS: We identified multiple univariate correlations between regional CNV status and spatially matched MRI texture features that were specific to either male or female GBM tumors. For instance, EGFR++ specifically correlated with T2W image textures in male biopsies but rCBV textures in female biopsies. In general, sex-specific analyses on decision-tree modeling improved predictive accuracies (AUC) compared to combined (male+female) modeling, particularly for EGFR++ ($p<0.05$), PTEN--($p<0.025$), and TP53-- ($p<0.025$). CONCLUSION: Sex-differences impact MRI-genetic correlations and warrant further study in larger GBM cohorts.

NIMG-13. SEGMENTATION AND VOLUMETRIC ANALYSIS IMPROVES DETECTION OF PROGRESSION IN LOW GRADE GLIOMAS

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Tumor surveillance is a common practice in oncology; its goal is timely detection of cancer progression, which is typically diagnosed by Visual Comparison (VC) or bidirectional measurement of longitudinal radiological images. Low-grade gliomas (LGG) can cause significant neurological morbidity by progressive brain invasion over time. We analyzed the longitudinal magnetic resonance imaging (MRI) studies of patients, diagnosed with grade 2 gliomas without any radiation therapy at the initial diagnosis, who had at least 4 MRI studies. Forty-eight patients met the inclusion criteria, including 13 oligodendrogliomas, 24 astrocytomas and 11 mixed gliomas. Thirty-four patients had clinical progression (CP) and 14 patients were considered clinically stable (CS) by VC at the time of the study. The computer assisted diagnosis (CAD) method consisted of segmentation of the fluid-attenuated inversion recovery (FLAIR) images, computing tumor volumes, and determining progression by the online abrupt change of point method, which considered only past MRIs. In the CP group, CAD detected progression at earlier times than the clinical diagnosis in 26/34 patients. In the CS group, CAD detected progression in 10/14 patients. Six physicians from the departments of neurology (neuro-oncology), radiology, and radiation oncology reviewed and agreed with the progression determined by the CAD method including the study subjects in the CS group. CAD was able to diagnose tumor progression when the tumor size grew at an average of 74% (range 14% to 364%) since the base line compared to 320% (range 25% to 2019%) in the VC method. The average time to progression was only 17 months when CAD was used compared to 50 months when VC method was used. Early detection of tumor progression is critical for LGG patients because it can optimize therapeutic options with significantly lower volume of brain resection or irradiation

NIMG-14. MULTIREGIONAL RADIOMICS PROFILING FROM MULTIPARAMETRIC MRI: IDENTIFYING AN IMAGING PREDICTOR OF IDH1 MUTATION STATUS IN GLIOBLASTOMA MULTIFORME

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Glioblastoma Multiforme (GBM) patients with isocitrate dehydrogenase 1 (IDH1) mutations have significantly improved prognosis than those without such mutations. We aimed to preoperatively predict IDH1 mutation status in GBM using multiregional radiomics features from multiparametric magnetic resonance imaging (MRI). In total 225 patients were recruited in this retrospective multicenter study. 1614 quantitative features were extracted from multiple tumor subregions (including enhancement area, non-enhancement area, necrosis and edema) in multiparametric MRI. After intensity normalization, tumor subregion segmentation, resampling-based data balancing and relevant feature selection, a multiregional radiomics model was built using a machine-learning method for prediction of IDH1 mutation from a primary cohort (118 patients) and tested on an independent validation cohort (107 patients). Four single-region radiomics models with features from each tumor subregion, and a model combining multiregional features with clinical factors (age, sex, and Karnofsky performance status) were also built and tested. Among four single-region radiomics models, the model built from edema region achieved the best accuracy of 96% and the best F1-score of 0.75 in the independent validation cohort. The 8-feature multiregional radiomics model achieved an improved overall performance of an accuracy 96%, an AUC 0.90 and an F1-score 0.78 in the validation cohort, which significantly outperformed the single-region models. Among all predictive models, the model combining multiregional imaging features with patient age achieved the best performance of an accuracy 97%, an AUC 0.96 and an F1-score 0.84 in the validation cohort. The radiomics-based model built with a minimal set of multiregional features from multiparametric MRI has the potential to preoperatively detect the IDH1 mutation status in GBM patients. The proposed predictor may serve as a potential noninvasive biomarker to guide preoperative GBM patient care. Keywords: Radiomics, IDH1 mutation, Multiregional imaging analysis, Glioblastoma multiforme

NIMG-15. DIFFERENTIATION OF IDH1 MUTANT AND WILD TYPE GLIOMAS USING pH- AND OXYGEN-SENSITIVE MOLECULAR MRI

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The presence of isocitrate dehydrogenase 1 (IDH1) gene mutation (IDH-1mut) in glioma patients is associated with longer survival compared to IDH1 wild-type (IDH1wt) patients. Non-invasive detection of IDH1 mutation could provide valuable diagnostic and prognostic information. Studies showed that IDH1mut correlated with decreased hypoxia-inducible-factor 1-alpha (HIF1α) expression and decreased level of glutamate. With a newly developed MRI technique using amine-CEST with spin-and-gradient echo EPI readout (CEST-SAGE-EPI), we simultaneously measure the pH-dependent chemical exchange effects and oxygen-sensitive reversible transverse relaxation rate (R_2'). In the current study we propose a novel imaging biomarker, which is sensitive to hypoxia, amine proton concentration, and tissue acidity, providing a potential non-invasive approach to detect IDH1 mutation and monitor related metabolic changes. In this study, 25 patients with glioma (8 WHO grade II: 2 IDH1wt/6 IDH1mut, 11 WHO grade III: 4 IDH1wt/7 IDH1mut, 6 WHO grade IV: 5 IDH1wt/1 IDH1mut) underwent whole brain CEST-SAGE-EPI scanning (7:30 minutes) and anatomic imaging on 3T MRI scanners. Stereotactic MRI-guided biopsies/tissue resections were performed in 10 of the 25 patients (29 samples), and immunohistochemistry (IHC) staining for HIF1a expression was analyzed. The results showed a significantly higher product of magnetization transfer asymmetry (MTR_{asym}) at 3.0ppm and R_2' in IDH1wt gliomas compared to IDH1mut gliomas (p=0.0033, Student's t-test). The significantly higher $MTR_{asym}xR_2$' persisted even when excluding grade IV gliomas (p=0.0063, Student's t-test). The ROC of differentiating IDH1 mutation status with $MTR_{asym}xR_2$' gave area under the curve (AUC) of 0.857 (sensitivity 85.71%, specificity 72.73%). The IHC staining of HIF1α showed a moderate positive correlation (Pearson's correlation R=0.439, p=0.0360) with $MTR_{asym}xR_2$'. The results demonstrated the potential of using imaging biomarker, $MTR_{asym}xR_2$', to detect IDH1 mutation status in gliomas, and to provide non-invasive information of tumor microenvironment.

NIMG-16. IMPACT OF SEX DIFFERENCES AND TUMOR LOCATION ON SURVIVAL OUTCOMES IN GLIOBLASTOMA PATIENTS

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INTRODUCTION: Glioblastoma (GBM) is the most common malignant primary brain tumor in adults with a median survival of 1416 months. Patient sex plays an important role in GBM as there is a difference in incidence rates and outcome between males and females, which may be attributable to differences in genetic makeup and physiology. OBJECTIVE: Investigate the impact of tumor location and sex differences on survival outcomes based on tumor location, laterality, age, handedness, and extent of resection. METHODS: Patients (129 males and 87 females) who received standard-of-care were included. Analyses were performed using Cox proportional hazard modeling and Kaplan-Meier analysis (log-rank test) to determine which variables impacted patient survival. RESULTS: Overall survival was significantly longer in females in comparison to males (197 days, p = 0.0391). Investigating specific tumor locations, females with a tumor in the left frontal lobe (n = 12) showed a survival advantage compared to females with a right frontal (n = 15) GBM (2853 days, p = 0.0160). Significant differences in median OS were also associated with age. Female patients below the age of fifty showed significantly longer survival (2602 days, n = 84, p<0.001). Interestingly, 70% of IDH1 mutant tumors (n = 10) and 76% of MGMT methylated tumors (n = 26) were found in the frontal lobe and were found in the right hemisphere. CONCLUSION: Together, our results demonstrate that age, sex, and specific brain locations are associated with differences in genetics and survival in GBM.

NIMG-17. UTILIZING MACHINE LEARNING FOR PREDICTIVE MODELING OF SEIZURE PRESENTATION IN GLIOMA PATIENTS

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PURPOSE: Seizures are frequent symptoms of gliomas. Predicting which patients are more likely to seize and in turn require anti-epileptic management has been a challenge. Correctly identifying these patients could help optimize care and minimize side effects. To provide guidance for clinicians, we used machine learning techniques to predict seizure presentation in this population. METHODS: We used volumetric data of pre-treatment MR images (T1Gd and T2-FLAIR sequences), patient demographics (age; sex), and measurements of tumor proliferation (log()), invasiveness (log(D)) and their relative ratio (log(/D)). We compared the performance of 5 machine learning models in predicting seizure status, using Artificial Neural Network, Naive Bayes (NB), Linear Discriminant Analysis (LDA), Random Forest, and Support Vector Machine. Correlations between probability of seizure presentation (p(SP)) and continuous variables were also analyzed. RESULTS: Our cohort consisted of 59 seizure-presenting and 77 non-seizure-presenting patients. All models consistently demonstrated significant correlations ($p < 0.05$) between (p(SP)) and the following variables: T1Gd radius (-0.781 to -0.674), T2-FLAIR (-0.674 to -0.611), and log(/D) (0.169 to 0.294). Age was significant ($p < 0.05$) in 4 of the 5 models (-0.211 to -0.175). Mean performance measures for the models (and the best performer) were: 0.726 for Area under the ROC curve (0.75 with NB), 0.6202 for sensitivity (0.661 with NB), 0.74 for specificity (0.766 with LDA). The 5 features ranked as most important were: T1Gd, T2-FLAIR, log(/D), age, log(). CONCLUSIONS: We found an association in seizure-presenting patients with smaller, more proliferative tumors and younger age. Machine learning predictive modeling can potentially be informative in the clinical arena. Further validation studies, to determine the degree of data overfitting, model versatility, as well as performance on test data, are warranted.

NIMG-19. SEX-SPECIFIC BRAIN MAPS FOR RISK OF SEIZURE AMONG GLIOMA PATIENTS

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PURPOSE: Despite a growing body of research on the etiology of seizures in the brain tumor population, it is not yet clear why approximately one third of this population presents with seizure. In this retrospective study we seek to assess the impact of tumor location on the risk of seizure among male and female glioma patients, respectively. METHODS: From our multi-institutional database, we selected adult patients with contrast-enhancing gliomas (any grade) and known seizure-presentation status at initial diagnosis. Tumors were segmented on pretreatment T2-FLAIR, T2, and post-gadolinium T1 (T1Gd) MR sequences. We warped cortical and subcortical probabilistic atlases to patient images and estimated tumor burden (%) on structures within each lobe and the deep brain. We calculated risk of seizure for various levels of tumor burden and used the result to create sex-specific risk-maps<./p> RESULTS: Our cohort included 128 patients (47 females, 81 males) among whom tumors presented with seizure in 34% of females (n=18) and 58% of males (n=47). Patients with tumors in deep brain structures were identified as at risk for seizure in both males and females. In female patients T2 hyperintensity on 44% of right frontal lobe or 28% of left parietal lobe seem to have an estimated 50%+ risk of seizure. Data indicate that in male patients with left-sided tumors, T2 hyperintensity on 30% or more of frontal, temporal, or parietal lobes results in 50%+ risk of seizure. Male patients with right side tumors, showed a similar level of risk for T2 presence in lateral ventricles and parietal lobe. CONCLUSION: This study reveals that the risk of seizure is specific to the location of glioma, the percentage of tumor burden, and patient sex. These differences could be considered in patient management decisions to more selectively prescribe anticonvulsants and optimize patient care.

NIMG-20. DIFFUSION-WEIGHTED MAGNETIC RESONANCE IMAGING FINDINGS IN PATIENTS WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT OF SYSTEMIC LYMPHOMA

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CNS involvement may be seen at any time point in patients with systemic Non-Hodgkin lymphoma (SNHL). The leptomeninges are the most common site of CNS involvement, however parenchymal metastases are seen in up to 1/3 of patients. Given the potentially disabling nature of such lesions, early diagnosis initiation of appropriate therapy are of paramount importance. MRI remains the primary tool for the initial detection of suspected CNS spread of systemic lymphoma. Diffusion-weighted imaging is often used employed in the diagnosis and response of cellular neoplasms. The primary goal of this study was to describe DW-Imaging characteristics in a retrospective cohort of patients with SNHL with nervous system involvement. We identified patients with SNHL and dissemination to the CNS parenchyma diagnosed at our institution between 2010 and 2018. MR data with the following sequences: T1 (with gadolinium administration), T2- FLAIR, DWI and ADC sequences were required for inclusion. Imaging characteristics such as number of lesions, location of lesions, presence of contrast-enhancement, ADC, DWI signal intensity were evaluated. Quantitative ADC analyses were performed within a target lesion, peritumoral edema, and normal white matter. Twenty-six patient fulfilled eligibility criteria. Data for quantitative analysis of ADC signal were available in 25 patients. All patients had enhancing disease. 24 of 26 patients (92%) had lesions with Hyperintense DWI signal and hypointense ADC signal. One-way ANOVA analysis showed significant difference between the mean ADC Lesion, ADC peritumoral region, and ADC white matter (f-statistic 142.79 and $p < 0.0001$). Post-Hoc analysis showed significant difference between ADC Lesion and ADC peritumoral region, ADC pertimuoral and ADC white matter, but not between ADC lesion and ADC white matter. DW-MRI is a useful adjunct to anatomical MRI sequences in the diagnosis of CNS dissemination of SNHL. In the appropriate clinical setting, comprehensive MRI analysis may obviate the need for biopsy confirmation.

NIMG-21. SEX DIFFERENCES IN EXTREME SURVIVORSHIP AMONG PRIMARY GLIOBLASTOMA PATIENTS

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Glioblastoma (GBM) is an aggressive primary brain cancer that has a median overall survival (OS) of 15 months from diagnosis. Determining which pretreatment tumor characteristics are predictive of extreme survival (EXS) (OS > 1825 days) and short-term survival (STS) (OS < 210 days) has significant clinical value. Several biological markers have been discovered that are predictive of EXS or STS, but the impact of patient sex on the predictive value of these markers has been minimally explored. In this investigation, machine learning algorithms and statistical tests were used to assess which pretreatment clinical and MR image-based volumetric and kinetic parameters were significant predictors of overall survival duration for male (n=299, including 30 EXS and 46 STS) and female (n=195, including 17 EXS and 42 STS) GBM patients. When compared to the middle survivor group (210 days < OS < 1825 days), female EXS had smaller tumor volumes and both male and female EXS had lower tumor cell proliferation rates at time of diagnosis. Independent predictors of overall survival included tumor cell diffuse invasion rate among females (hazard ratio [HR] =1.011, $p < 0.001$), tumor size among males (HR=1.027, p=0.044) and age among both males and females (Females: HR=1.021, p=0.006; Males: HR=1.030, $p < 0.001$). Despite similar distributions of the MR imaging parameters between males and females, there was a sex-specific difference in how these parameters related to outcomes, which emphasizes the importance of considering sex as a biological factor when determining patient prognosis and treatment approach.

NIMG-22. HIGH-GRADE GLIOMA OUTCOMES IN THE PHASE 1 BXQ-350 TRIAL OF CANCER-SELECTIVE SapC-DOPS NANOVESICLES

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BACKGROUND: Saposin C-dioleylphosphatidylserine (SapC-DOPS) is a novel blood-brain barrier penetrant nanoliposomal agent that targets externalized phosphatidylserine overexpressed on tumor cell membranes. A recent Phase 1a BXQ-350 trial (NCT02859857) reported SapC-DOPS was well tolerated in both solid tumor and high-grade glioma (HGG) patients with potential for treating clinically challenging gliomas, including their diffuse infiltrative components. METHODS: We evaluated the HGG subset of the ongoing Phase 1 study of IV BXQ-350 administered on Days 1–5, 8, 10, 12, 15, 22 (cycle 1) and each 28 day cycles thereafter. MRI neuroimaging (e.g. Axial T1-post-contrast Ax SE T1 POST-FC and medically necessary views) at Days 29, 57, 113, and 171 (or withdrawal) were completed. RANO assessment, functional neurological deficits, ECOG Performance Status, and safety were assessed. RESULTS: The HGG patients (9/17) were dosed at 0.7 (N = 1), 1.1 (N = 1), 1.4 (N = 2), 1.8 (N = 2), or 2.4 (N = 3) mg/kg, with 8/9 completing a full cycle before withdrawal (7 due to progression, 1 voluntary withdrawal). BXQ-350 was not linked to dose limiting toxicities or severe adverse reactions. Functional neurological deficits and ECOG decline were proportional to radiological progression, with ECOG scores declining from a baseline 0–1 in 2/9 (22%) to 3. One patient completing 6 cycles (>12 months) of BXQ-350 therapy (0.7 mg/kg) exhibited stable disease, -7% lesion size, and no significant progressive functional neurological deficits. Three patients underwent surgery for progression while on treatment. CONCLUSIONS: BXQ-350 was well tolerated by GBM patients with promising best response apparent in neuroimaging, suggesting therapeutic benefit warranting further trials. Updates from the ongoing trial will be presented.

NIMG-23. DEEP LEARNING FOR ACCURATE, RAPID, FULLY AUTOMATIC MEASUREMENT OF BRAIN TUMOR-ASSOCIATED ABNORMALITY SEEN ON MRI

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INTRODUCTION: Brain tumors are difficult to segment in MRI scans. Consequently, we have developed a new system for completely automatic brain tumor segmentation by combining a state-of-the-art 3D deep convolutional neural network (CNN) with a large collection of curated segmentations of brain tumors. METHODS: Our brain tumor database holds 74,722 MRI series from 2,742 unique patients. Over the last 15 years our image analysis team has segmented brain tumors in 35,710 of these series. This preliminary experiment identified 741 pre-treatment studies that included a T1GD and FLAIR scan, and at least one adjudicated brain tumor segmentation. These studies were randomly assigned into 600 training, 41 validation, and 100 test cases. CNN training was performed in two stages: 1) 50 epochs on minimally modified MRI volumes; and, 2) 24 epochs to tune the CNN on skull-stripped volumes. Whole-tumor Dice coefficients (1=perfect overlap, 0=no overlap) were calculated by comparing CNN segmentations against adjudicated segmentations from trained measurers. Training was performed in-the-cloud using an Amazon Machine Instance equipped with an NVidia Tesla V100 GPU, 8 Intel Xeon processors, and 64 GB of RAM. RESULTS: Training required 74 hours. Afterwards, our network required 800 seconds to segment 100 studies in the test set (8 seconds/study). The mean whole-tumor Dice coefficient on the test studies was 0.885. DISCUSSION: The best result on the highly cited 2017 BraTS brain tumor segmentation challenge was a whole-tumor Dice of 0.886, achieved by an ensemble of 7 CNNs. BraTS included 274 studies, each with T1GD, FLAIR, T1 and T2 contrasts. The performance of our single CNN may be due to our

comparatively large training set. Our goal is to train our CNN on all series in our database. This may provide useful tools to monitor each patients journey, from diagnosis through treatment.

NIMG-24. DIFFUSION MRI PRECURSORS TO PROGRESSION IN GRADE II AND III GLIOMA

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The goal of this project is to identify serial advanced imaging markers that can reveal tumor progression in grade II and III gliomas prior to the to the onset of new contrast enhancement or the expansion of FLAIR abnormality. Nineteen lower grade glioma patients were identified with tumor progression during their participation in research on serial advanced MR imaging and spectroscopy at standard of care timepoints (2–6 months). The average number of scans acquired prior to progression was 7 (range = 12–3). The 3T MRI examination included T1-weighted IRSPGR, T2-weighted FSE, T2 FLAIR, and 6 directional diffusion weighted imaging. Maps of the apparent diffusion coefficient (ADC) and fractional anisotropy (FA) were calculated, and percentiles (10^{th}, 50^{th}, 90^{th}) from histograms of normalized intensities. Serial images were aligned to the baseline image. Regions of Interest (ROIs) were defined for the entire T2 FLAIR lesion, and when present, the contrast-enhancing (CE) T1 lesion at each timepoint. In addition, we created a Progression ROI consisting of the region that was normal on the baseline T2 FLAIR but abnormal at the time of clinical progression, and a Baseline ROI consisting of the region that was abnormal at baseline and progression. Serial imaging parameters were acquired for each of these ROIs at every timepoint. We determined the imaging parameters with significant changes within the ROIs leading up to the time of progression using repeated-measures analyses of variance. Leading up to the time of progression, there were significant declines in median and 10% normalized ADC and 10% normalized FA within the Baseline T2 FLAIR abnormality ROI. Median normalized ADC also declined significantly in the Progression ROI, indicating changes in diffusion parameters prior to the onset of T2 FLAIR abnormality and the clinical determination of progression.

NIMG-25. MOLECULAR PHYSIOLOGY OF CONTRAST ENHANCEMENT IN GLIOBLASTOMAS: AN ANALYSIS OF THE CANCER IMAGING ARCHIVE (TCIA)

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The biologic processes that contribute to contrast enhancement on magnetic resonance imaging of glioblastoma patients remain poorly understood. Glioblastoma tumors from The Cancer Imaging Archive were segmented using iterative probabilistic voxel labeling. Three parameters of contrast enhancement (CE) were calculated: intensity (CE_i), heterogeneity (CE_h), and volumetric ratio to necrosis (CE_r). Associations between these parameters and gene expression from The Cancer Genome Atlas were examined to gain insights into their underlying biologic process. Glioma CpG island methylator phenotype glioblastomas (G-CIMP) were poorly enhancing. No differences in CE parameters were found between proneural, neural, mesenchymal, and classical glioblastomas. Increased CE_i was associated with expression of genes that mediate inflammatory immune responses. High CE_h was associated with increased genes required for tumor migration and invasion, including those that modulate extracellular matrix (ECM) and endothelial vascularity. High CE_r was associated increased gene expression associated with stressful metabolic states, including hypoxia and starvation. Our results indicate aspects of CE are associated with distinct underlying biology. Integrative analysis of these CE parameters may yield meaningful information pertaining to the biologic state of glioblastomas and guide future therapeutic paradigms.

NIMG-26. RADIOMIC FEATURES OF GLIOBLASTOMA ON PRE-TREATMENT GD-T1W MRI ARE PREDICTIVE OF RESPONSE TO CHEMO-RADIATION THERAPY AND ASSOCIATED WITH AKT AND APOPTOSIS PATHWAYS

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BACKGROUND: >40% of Glioblastoma patients do not respond to chemo-radiation (Ch-Rx) treatment and recur within 6–8 months, suggesting that they could be better candidates for experimental therapies. We seek to discover radiomic features on pre-treatment MRI that are predictive of response to Ch-Rx. Further, in an attempt to establish biological underpinning of these predictive radiomic features, we then identified radiogenomic correlations between these features with molecular pathways that are known to impact response to Ch-Rx. METHODS: 49 GBM studies were obtained from publicly available IVYGap (n=29) and TCIA (n=20) databases, with pre-treatment MRI scans (Gd-T1w, T2w, FLAIR) and corresponding RNA-Seq data. Responders were defined as patients with progression-free survival (PFS) of >= 4-months, while non-responders had PFS of <4-months. A total of 1305 3D-radiomic features (Gabor, Haralick, and Laws energy) were extracted from each MRI protocol from expert annotated regions (enhancing, edema/non-enhancing, necrosis) for every study. Top 5 predictive features were obtained from a training set and validated using a support vector machine classifier. 13 signaling pathways that are known to be implicated in Ch-Rx response were curated from the MSigDB HALLMARK cohort. Gene Set Enrichment Analysis (GSEA) scores of these pathways was correlated with the top radiomic features with Bonferroni correction. RESULTS: Laws energy features that characterize appearance of ripples and spots from enhancing region on Gd-T1 MRI were found to best predict Ch-Rx response (sensitivity of 73.3% on validation set). These features were statistically significantly correlated with AKT and apoptosis signaling pathways ($p<0.02$, FDR = 5%). CONCLUSION: Activation of AKT pathway facilitates angiogenesis and develops Ch-Rx resistance. Similarly, higher expression of apoptotic proteins in GBM is associated with favorable Ch-Rx response. Our radiogenomic approach may allow for non-invasive CRT response prediction by providing biological understanding of molecular pathways implicated in Ch-Rx response, as manifested on pre-treatment Gd-T1 MRI.

NIMG-27. RADIOGENOMIC ANALYSIS OF GLIOBLASTOMA REVEALS TEXTURAL FEATURES FROM MRI THAT CORRELATE WITH GENOMIC IMMUNE SCORE AND ARE ALSO PREDICTIVE OF CHEMO-RADIATION TREATMENT RESPONSE

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BACKGROUND: Elevated immune response in Glioblastoma (GBM) is known to improve chemo-radiation therapy (CRT) response. Understanding this manifestation at a radiologic scale may establish non-invasive features as potential predictive biomarkers. We seek to discover pre-treatment radiomic MRI features associated with immune response, and further evaluate their capability to predict CRT response. METHODS: 74 GBM patients from TCIA and TCGA with pre-treatment MRI and corresponding RNA sequencing prior to CRT were retrospectively analyzed. Immune score (IS) was derived from a 140-gene immune signature via the ESTIMATE algorithm. 1400 3D-radiomic features (Gabor, Haralick, Laws) were extracted from T1w, T2w, FLAIR within expert annotated regions (enhancing tumor, edema, necrosis) per patient. Least absolute shrinkage and selection operator (LASSO) was performed in 100 iterations of 3-fold cross-validation on the training set (n=40) with respect to IS. The 4 most selected radiomic features were used to derive a multilinear regression model of IS. This model was applied to predict IS within an independent set (n=34). Within test set, responders (R) were defined as patients with progression-free survival (PFS) of >= 4-months, while non-responders (NR) had PFS of <4-months. Unsupervised clustering of radiomic features was then implemented to categorize every study as R (n=18) or NR (n=16) to CRT. RESULTS: The radiomic model was significantly associated with IS in training ($R2=.61$, $p<.0001$) and testing ($R2=.16$, $p=.018$) sets. Top IS-associated features captured wave-like enhancement patterns and intensity value heterogeneity within the tumor or peritumoral edema on T1w and T2w. Within the testing set, unsupervised clustering yielded accuracy=.71 (Sensitivity=0.78, Specificity=.63) in distinguishing R from NR. CONCLUSION: Elevated IS, indicative of a robust immune response preceding treatment, may manifest as subtle changes in peritumoral edema detectable on pre-treatment MRI. Radiomic correlates of IS may provide means of quantifying immune activity and help predict response to CRT.

NIMG-28. CONVENTIONAL AND ADVANCED IMAGING FINDINGS FOLLOWING INTRACAVITARY DELIVERY OF AUTOLOGOUS CHIMERIC ANTIGEN RECEPTOR T-CELL THERAPY IN RECURRENT GLIOBLASTOMA

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INTRODUCTION: Chimeric antigen receptor (CAR) T-cell therapy is an emerging area of immunotherapy for treatment of recurrent glioblastoma (GBM). However, evaluating response to CAR T-cell therapy is a consider-

able challenge. Radiologic changes following CAR T-cell therapy may include inflammation, which may be misinterpreted as disease progression, resulting in premature termination of the treatment. The iRANO criteria provides a 'limbo window' of up to six months from the start of immunotherapy during which radiologic worsening is tolerated while continuing immunotherapy. However, the time frame for identifying tumor progression following CAR T-cell therapy in the recurrent setting has not yet been characterized. AIM: Here we report imaging findings from conventional and advanced MRI sequences along with 18-FDG-PET-CT, acquired before, during, and after intracavitary administration of autologous central memory derived IL13Rα2-targeted CAR T-cell therapy for recurrent glioblastoma. METHODS: Imaging findings are reported from an ongoing dose-escalation study (NCT02208362) with maximum doses of $10x10^6$ (dose schedule 1), $50x10^6$ (dose schedule 2) and $100x10^6$ (dose schedule 3) CAR T-cells. All patients underwent MRI scans on a 3T Siemens scanner prior to surgical debulking and CAR T-cell administration as well as at the end of each CAR T-cell treatment cycle, consisting of 3 CAR T-cell infusions into the resection cavity corresponding to time intervals of approximately 1 month. Conventional anatomic MRI included T1-weighted pre- and post-contrast, T2-spin echo, and T2-FLAIR sequences. Quantitative MRI included Dynamic Contrast Enhanced (DCE) Perfusion MRI and Diffusion Weighted Imaging (DWI). Additionally, patients underwent functional metabolic imaging with 18-FDG-PET-CT. Multiparametric imaging characteristics will be compared longitudinally before, during, and following CAR T-cell treatment and correlated to clinical progression. As of May 2018, 14 patients have been treated: dose 1 (n=3), dose 2 (n=3), dose 3 (n=8). Findings and analysis will be updated at the time of late-breaking abstract deadline.

NIMG-29. IN SILLICO BRAIN TUMOR MODELS FOR VALIDATING NEW DYNAMIC MR IMAGING METHODS PRIOR TO CLINICAL TRIALS

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New dynamic MRI methods hold promise in better characterization of the complex structure and heterogeneous treatment response of brain tumors. These methods utilize assumptions to reduce the data samples needed to produce high temporal and spatial resolution images with complex, iterative reconstructions. Determining how these methods depict actual brain tumor pathology is difficult due to lack of a gold standard. We therefore tailor an existing digital tool, built originally to simulate dynamic MRI breast studies, to assess the performance of new dynamic methods if applied to brain cancer. The tool permits a tumor of customizable shape featuring an inner core and an adjustable surrounding rim to be overlaid on a digital 3-D brain model, which we acquired from an online database called BrainWeb, with segmented brain tissue layers. We used existing capabilities in the tool to assign pharmacokinetic parameters to each tumor region to simulate necrotic cores and rims of varying width that represent spatially varying levels of enhancement due to tumor pathology and/or radiation necrosis. The tool then computes the corresponding MR k-space data for any proposed MR sampling trajectory during a simulated passage of a contrast agent. The simulated MR k-space data can then be input to any proposed reconstruction to produce a simulated time course of image volumes. Performance of proposed dynamic MR imaging methods can be measured against the digital truth prior to clinical trials through methods including: the structural similarity (SSIM) index over the lesion region-of-interest, comparing calculated vs. assigned pharmacokinetic parameters, and root-mean-square error. We are using this tool to estimate the performance, in brain applications, of a 3-D radial acquisition and reconstruction method with compressed sensing and local low rank that was previously demonstrated to produce 0.8 mm resolution in a 10 second frame rate in bilateral breast screening.

NIMG-30. PREOPERATIVE PREDICTORS OF MALIGNANCY IN NON-ENHANCING GLIOMA IN THE ERA OF MOLECULAR CLASSIFICATION

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INTRODUCTION: The association of contrast enhancement with malignancy in glioma is widely accepted. A higher grade of uncertainty exists for preoperative grading of non-enhancing tumors. Here, we sought to reevaluate tumor grading of non-enhancing glioma with the new WHO classification of 2016 and analyzed clinical data and radiographic features (T2/FLAIR mismatch sign, subventricular zone involvement, tumor volume and growth) which might predict WHO grading, IDH mutation or PFS. METHODS: Out of 626 glioma patients, 72 with non-enhancing glioma underwent supratentorial surgery in our department (2012 – 2017). Median follow-up was 24.5 months. Histopathological and molecular examinations were performed by our neuropathologists in concordance with the 2016 WHO classification. The "T2/FLAIR mismatch sign" and tumor involvement with the subventricular zone were evaluated by two independent investigators. Medical records of all patients were reviewed. The "Pignatti" score was calculated as previously described. RESULTS: 57% (41) of patients were IDHmut, 43% (31) IDHwt. 75% of the total study cohort (54 patients) in which preop-MRI suggested low grade glioma, were classified grade III or IV and thus considered malignant. Involvement of the subventricular zone correlated with PFS (log-rank p= 0.02). Age significantly correlated with PFS (log-rank p= 0.01) and with tumor grade (log-regression p< 0.001). When applying the new WHO classification to the Pignatti score, no correlation in PFS and tumor grading could be seen. The T2/FLAIR mismatch sign was confirmed to be highly specific for IDH mutation (positive predictive value 96%). CONCLUSION: 75% of non-enhancing suspected low grade glioma were indeed grade III or IV according to the new WHO classification of 2016. We confirmed the T2/FLAIR-mismatch sign to be a highly specific marker for IDH mutation and found that involvement of the tumor with the subventricular zone was significantly correlated with worse PFS. These results may help to improve preoperative patient counseling.

NIMG-31. BREAST CANCER SUBTYPE AS A DETERMINANT OF THE INTRACRANIAL METASTASES LOCATION

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The frequency of brain metastases (BM) in patients with metastatic breast cancer is up to 10–24% during the course of their disease. Studies have shown that the metastatic behavior is correlated to the sub-type of the breast cancer. Breast cancer sub-types are determined by the gene expression of the cancer cells. The five groups are luminal A [estrogen receptor (ER /PR+ HER2-)]; luminal B (ER /PR+ HER2+); HER2 over-expressing; normal breast-like; and Triple negative (TN) carcinomas basal-like. Patients with TN and HER2+ tumors are at higher risk of BM. We hypothesize that the genetic sub-type that influence how the breast cancer metastasizes can also have implications in where the metastasis occurs. An IRB approved retrospective review of the medical records of 50 patients referred from the radiation oncology with breast cancer BM in our institution spanning April 2006 to April 2018. Patients were divided into groups according to the age, location of the intracranial metastases, breast cancer sub-type, primary tumor stage, Ki-67 proliferation marker, previous chemotherapy. The breast cancer sub-types were recorded from the pathology reports. The location of the intracranial metastases was done by reviewing the first MRI brain study with positive metastases; the findings on the post contrast fat suppressed axial T1 weighted images and the study date were recorded. Sixteen patients were ER/PR+ HER2-, thirteen were ER/PR+ HER2+, eighteen were TN and 3 HER2 over-expressing. The cerebellum metastases were observed in twenty-eight patients. Eleven of the twenty-eight were of ER/PR+ HER2+, ten were TN, five were ER/PR+ HER2- and 2 were HER2 over-expressing. By using the Chi-square statistical analysis there was a significant association between the ER/PR+ HER2+ sub-type and the presence of cerebellar metastasis ($p<0.05$). We conclude that breast cancer BM expressing ER/PR+ HER2+ have preferential cerebellar involvement.

NIMG-32. COMPARISON OF L-METHYL-11C-METHIONINE POSITRON EMISSION TOMOGRAPHY WITH MAGNETIC RESONANCE SPECTROSCOPY IN DETECTING NEWLY DIAGNOSED GLIOMA

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BACKGROUND: Amino acid positron emission tomography (PET) and magnetic resonance spectroscopy (MRS) are at the forefront of non-invasive imaging techniques used for detection and subtyping of glioma-suspicious lesions. No reported studies so far attempted to compare those techniques performed during one single preoperative session in their ability to predict glioma subtypes. METHODS: Twenty patients with histologically confirmed newly diagnosed glioma underwent preoperative MET PET and MRS during one single diagnostic session. According to the molecular portfolio and histopathological diagnosis, patients were subdivided in isocitrate dehydrogenase (IDH) wildtype glioblastoma (GBM), IDH wildtype grade II/III glioma (IDHW), IDH mutant grade II/III glioma without 1p/19q codeletion (IDHMnc) and with 1p/19q codeletion (IDHMcod). Maximum tumor-to-brain ratio (TBRmax), creatine, choline and N-acetyl aspartate (NAA) peaks were correlated with postoperative histopathological tumor diagnoses. To gain generalizable implications from our data we subdivided the full study cohort into a development and validation subcohort. A support vector machine model was fitted to the development subcohort and evaluated on the validation subcohort. Receiver operating characteristic (ROC) curve with area under the curve (AUC) as metric served to assess model performance. RESULTS: TBRmax was highest in GBM patients (4.18), followed by IDHW patients (3.04). The latter TBRmax values were higher compared to those in IDHMnc patients (1.95) and in IDHMcod patients (2.79). MRS marker distribution showed no clear trend. ROC analysis revealed TBRmax to be the best performing parameter in identifying IDH status (AUC: 0.67) and all spectroscopy markers combined in identifying glioma subgroups (AUC: 0.68). CONCLUSIONS: MET PET and MRS bear limited potential in glioma subgrouping. However, MET PET appears to be slightly superior in differentiating IDH status. Investigation in a larger cohort is required to draw definite conclusions.

NIMG-33. MULTICENTER, PROSPECTIVE VALIDATION OF AUTOMATED INTRAOPERATIVE NEUROPATHOLOGY USING STIMULATED RAMAN HISTOLOGY AND CONVOLUTIONAL NEURAL NETWORKS

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INTRODUCTION: Accurate intraoperative diagnosis is essential for providing optimal neurosurgical care. In many centers caring for brain tumor patients, neuropathology resources are limited. To augment existing neuropathology resources, we developed and validated a new paradigm combining optical histology and artificial intelligence (AI) to accurately predict diagnosis during brain tumor surgery. METHODS: A total of 1026 specimens from 501 patients undergoing brain tumor resection at two tertiary hospitals were imaged using an optical technique, called stimulated Raman histology (SRH). SRH images were used to train and validate a convolutional neural network (CNN) for state-of-the-art computer vision. We redesigned the GoogleNet InceptionV3 CNN architecture to optimize performance on SRH histologic image fields of view (FOVs) and trained the network using 466 patients (3.1 million unique 300m2 FOVs) to classify into 13 common brain tumor subtypes. Final intraoperative diagnosis was determined using the most commonly predicted FOV diagnosis within each specimen. Model testing was completed on 1 million unique FOVs from 35 prospectively enrolled patients whose data was not included in the training set. RESULTS: In the validation set, our trained CNN differentiated lesional from normal tissue with 100% accuracy, surgical from nonsurgical lesions with 100% accuracy, glial from non-glial tumors with 100% accuracy. When evaluating our model for tumor subtype classification, we achieved an accuracy of 97% (35/36 patients) compared to final clinical diagnosis. Corresponding clinical frozen section diagnostic accuracy was 97% and interrater agreement between CNN and clinical frozen section diagnosis was near-perfect (k>0.96). The sole CNN error was misclassification of a WHO grade 1 pilocytic astrocytoma as a WHO grade II astrocytoma. CONCLUSION: Our prospective, multi-institutional validation suggests that AI can be applied to predict diagnosis in neurosurgical specimens in an automated fashion. AI-based diagnosis may ultimately be used to augment the current neuropathology workflow where resources are limited.

NIMG-34. THE IMPACT OF TUMOR TREATING FIELDS (TTFIELDS) ON BRAIN ANATOMY USING COMPUTATIONAL ANATOMY ANALYSIS

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BACKGROUND: TTFields is a glioblastoma (GBM) treatment based on focal electric field stimulation that inhibits tumor progression by disrupting cell division. In this pilot study, we sought to use computational anatomy analysis and quantitative magnetic resonance imaging (MRI) data to evaluate treatment effects over time looking at both the effects on the tumor tissue and normal brain parenchyma. METHODS: MRI data were acquired at 3-month intervals before and during TTFields treatment in patients with glioblastoma (currently 10 patients enrolled, recruitment ongoing). The whole-brain MRI quantitative protocol comprises three multi-echo 3D fast low angle shot (FLASH) acquisitions with predominantly magnetization transfer-, proton density- and T1-weighted contrast. For statistical analysis, all MT images were classified by tissue classes: grey matter, white matter, cerebral-spinal fluid and non-brain tissue. All maps were warped in standardized space and then smoothed by convolution with an isotropic Gaussian kernel. Regional differences were examined in a paired t-test creating voxel-wise statistical parametric maps (SPMs). RESULTS: In whole-brain SPM analysis we observed a decrease in macromolecular and iron content confined to the tumor-affected area and corpus callosum between the pre-treatment and post-treatment time points. No changes were identified in the non-affected hemisphere. CONCLUSIONS: These preliminary results show the potential of quantitative MRI as a biomarker sensitive to tissue changes to monitor TTFields or other treatment effects over time. We underscore the quantitative character of our data that renders them virtually free of time-related bias and the ability to provide a biological interpretation of the results linking them to macromolecular and iron content. Given the relative spatial heterogeneity of brain tumor location in our patients we attribute the paucity of significant results to common brain tissue property changes going beyond individual lesion spread. Future analyses will focus on within-subject analysis across time.

NIMG-35. TREATMENT RESPONSE ASSESSMENT MAPS (TRAMs) SENSITIVITY TO TUMOR/TREATMENT-EFFECTS AS A FUNCTION OF DATA ACQUISITION PARAMETERS

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INTRODUCTION: TRAMs calculated from delayed-contrast MRI enable reliable (sensitivity/specificity>70%) differentiation between tumor (blue in the TRAMs) and non-tumoral tissues (red). The TRAMs are calculated by subtracting 3D T1-MRIs acquired 5min (early time point) post-contrast injection from those acquired 60-105min (late point) later. Here we studied the sensitivity to tumor/treatment-effects as a function of the early T1-MRI acquisition time. METHODS: 7 patients with high grade glioma and 6 with brain metastases were scanned by the standard TRAMs protocol with the addition of a rapid 3D T1-MRI sequence (20 sec) acquired 2, 5, 12, 17, 20, 24 and 70 min post-contrast. Rapid-TRAMs were calculated using the rapid T1-MRIs, where the late time point was fixed at 70 min and the early time point changed from 2 to 24 min post-contrast. Enhancing volumes were determined on the T1-MRIs and copied to the TRAMs. Blue/tumor and red/treatment-effects volumes were calculated within the enhancing regions. RESULTS: The blue/tumor volumes, calculated from the rapid-TRAMs, increased by a factor of 4.4 ± 2.6 when moving the early time point from 2min to 15.7 ± 2.2min, where they plateaued. The increase between 5min (standard) and 15.7min was by 1.5 ± 0.3. In contrast, when moving from 2 min to 15.7min the red/treatment-effects volumes decreased by 0.7 ± 0.2, and by 0.8 ± 0.1 when moving from 5min. CONCLUSIONS: The TRAMs were shown to provide reliable differentiation between tumor/treatment-effects. The early time point is fixed at 5min post-contrast. Using shorter delays may significantly decrease the sensitivity to tumor. Still, increasing the delay to 15min may increase the sensitivity to tumor. This over-estimation of the tumor volume may be explained by the tumor vasculature clearing contrast diffusing into further brain regions surrounding the tumor. An additional 3D-T1 acquired at 15min may be applied for calculating additional TRAMs with higher sensitivity to tumor, for depicting small tumor regions.

NIMG-36. THE SIGNIFICANCE OF PUNCTATE CONTRAST-ENHANCING LESIONS IN TREATED HIGH GRADE GLIOMA

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We report on a series of nine patients with high grade glioma (HGG) who developed punctate contrast-enhancing foci on MRI, several years after tumor treatment. The lesions were considered suspicious for tumor progression at their onset, but were proven over time to be benign in nature. Five of the patients were treated for glioblastoma; 3 for anaplastic astrocytoma, and one for anaplastic oligodendroglioma. Median age of the patients at diagnosis was 48 years (31–62 years). The mean time of onset of "suspicious spots" following treatment was 51 months after diagnosis (6–170 months). Of note, median overall survival of the group is 96 months; with none of the nine patients deceased, and only 2 patients known to have progression. The remainder of the 7 patients are free of active tumor and being followed off treatment. No apparent clinical manifestations of the lesions have been observed. The majority of the punctate lesions were of a dynamic nature; they appeared and spontaneously disappeared over time, without intervention. All lesions developed within the field of radiation treatment; some were contralateral to the tumor. Radiological characteristics of the lesions including analysis of SWI, DWI, and DCE magnetic resonance imaging sequences will be reported. Punctate enhancing foci in the region of radiation treatment are phenomena which may be manifestation of delayed treatment sequelae in HGG. Patients with these lesions are on average of younger age and have a particularly long survival. These lesions appeared several years following oncological treatment. The foci should be initially followed more closely until shown to be of a non-progressive nature; they should not be presumed to be indicative of tumor recurrence. Possible etiology of the lesions may be related to vascular changes induced by radiation.

NIMG-37. ASSOCIATION BETWEEN METABOLIC PARAMETERS FROM DYNAMIC 18FMC PET, PHARMACOKINETIC DCE-MRI PARAMETERS, MRS CHOLINE TO CREATINE RATIOS AND TISSUE IMMUNOHISTOCHEMISTRY FOR CHOLINE KINASE ALPHA EXPRESSION IN HUMAN BRAIN GLIOMA

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INTRODUCTION: Proton MR spectroscopy and Choline-PET probe different aspects of choline metabolism, and quantitative dynamic MRI yields information on vascular permeability and perfusion. The relationship between these features in different grades of glioma is, however, unclear. METHOD: 14 patients with suspected primary supratentorial glioma were recruited to this study. The mean values over the whole tumour (T2-FLAIR hyperintense regions) of DCE-derived pharmacokinetic parameters were correlated with tumour to background ratio (TBR: ratio of SUVmax in tumour to SUVmean in contralateral white matter for the 7-17-minute static PET images). Dynamic PET macroparameters were quantified with spectral analysis (SA) in six patients for whom metabolite data were available. Choline to creatine ratios (Cho/Cr) were extracted from 2D-CSI data over 257 MRS voxels and correlated with TBR. Tissue immunohistochemistry for choline kinase alpha expression in targeted biopsies was carried out in regions of tumour with high and low uptake on PET and Cho/Cr on MRS. RESULTS: We observed a positive significant correlation between DCE-MRI derived parameters and parameters obtained through SA of the dynamic choline-PET data as well as TBR. We also observed a positive significant correlation between MRS Cho/Cr and TBR, although this was weak when excluding WHO Grade IV tumours. We did not observe a strong correlation between choline markers on imaging and choline kinase alpha expression. CONCLUSION: The correlation between both DCE and MRS parameters with TBR indicates that a number of biological features affect the uptake of the PET tracer. DCE-MRI provides complimentary information to blood volume and permeability that may augment interpretation of PET data; and help address questions such as the degree to which tracer uptake is dominated by blood brain barrier permeability rather than metabolic activity. Choline imaging with PET and MRS may reflect metabolic processes that are not simply related to choline kinase alpha expression.

NIMG-38. QUANTITATIVE IMAGING PREDICTORS OF OVERALL SURVIVAL IN GLIOBLASTOMA PATIENTS ROBUST IN THE PRESENCE OF INTER-SCANNER VARIATIONS

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BACKGROUND: Glioblastoma is the most aggressive primary adult brain tumor with median overall survival (OS) of ~14 months following treatment. Although associations have been shown between multi-parametric magnetic resonance imaging (mpMRI) signatures and OS in glioblastoma patients, there is no sufficient validation across different institutions/scanners. This study explores appropriate normalization approaches and multivariate machine learning (ML) to identify robust and reproducible imaging predictors of OS across scanners/institutions. METHODS: We identified a retrospective cohort of 208 patients, who underwent surgery with gross total resection status, and had available mpMRI (T1,T1-Gd,T2,T2-FLAIR,DTI,DSC) data from three different scanners in the Hospital of the University of Pennsylvania. Median OS was used as a cut-off between long- and short-survivors. Inter-scanner harmonization of mpMRI was conducted by normalizing the tumor intensity profile, with that of the contralateral healthy tissue. Intensity distributions, morphological, statistical, and texture descriptors extracted from intensity-normalized tumor sub-regions (enhancing, non-enhancing, edematous), were multivariately integrated via ML to derive predictors of patient OS. The predictors generalizability on unseen patient data was evaluated under two configurations: i) pooled/scanner-agnostic using 10-fold cross-validation, and ii) across scanners (training in multiple scanners and testing in one). RESULTS: The accuracy for predicting long- versus short-survivors was 80.64% (sensitivity=82.82%, specificity=78.16%, area under the curve [AUC]=0.79) and 75.26% (sensitivity=80.80%, specificity=68.97%, AUC=0.75) for pooled/scanner-agnostic and across-scanner configuration, respectively. The short-survivors, compared to long-survivors, showed relatively large and irregular infiltrating tumor, increased and compromised tumor microvascularity (DSC, T1-Gd), lower water concentration (T2), and higher cell density (DTI). CONCLUSION: Our findings suggest that quantitative analysis of appropriately normalized clinically-acquired mpMRI, coupled with ML, yields non-invasive robust predictors of OS for glioblastoma patients in presence of inter-scanner variations. Further validation of our predictors in more extensive multi-institutional datasets, can facilitate their potential incorporation into clinical practice, influencing surgical decision-making, treatment planning, and assisting patient management.

NIMG-39. THE EVALUATION OF DIFFUSION TENSOR TRACTOGRAPHY USING MIXED REALITY INTEGRATED VIRTUAL SPACE AND REAL SPACE

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PURPOSE: Diffusion tensor tractography (DTT) is a method to estimate the direction of white matter fibers, but it is not easy to verify the relationship with brain functions accurately. We developed mixed reality computer graphics (MRCG) which integrates real space (brain surface image) and preoperative 3DCG (3-dimentional computer graphics). In our previous study, this method allowed high precision registration even after brain shift caused by craniotomy, and target registration error was 0.5 ± 0.04mm (mean±SE). We devised a method to investigate the spatial error of DTT and direct cortical stimulation (DCS) using MRCG. We examine its accuracy and clinical application. METHODS: We covered 4 patients with glioma who were underwent awake surgery. The sex of all the patients was male, and the average age was 40. MRCG was constructed by fusing 3DCG prepared before surgery and brain surface photograph (JPEG format) just after craniotomy using our original method, which is called thin plate spline method with some landmarks. Brain function mapping results by DCS were plotted on MRCG. In the surface photograph, we provided grids of 1cm square, then we jagged the site where positive symptoms appeared by DCS was true, and the negative site was false. The spatial error between the cortical cortex and brain function mapping estimated by arcuate fiber of DTT was measured. RESULTS: In 4 cases, DCS was performed in 36 places (average 9 places / case). The sensitivity and specificity of DTT were 0.21 and 0.68, false positive rate and false negative rate were 0.31 and 0.79, respectively. In surgery, the tumor resection was performed with reference to MRCG reflecting the brain function mapping result. CONCLUSION: The proposed method investigated at real space and virtual space suggests DTT does not necessarily match the results of DCS.

NIMG-40. NON-INVASIVE IN VIVO SIGNATURE OF IDH1 MUTATIONAL STATUS IN HIGH GRADE GLIOMA, FROM CLINICALLY-ACQUIRED MULTI-PARAMETRIC MAGNETIC RESONANCE IMAGING, USING MULTIVARIATE MACHINE LEARNING

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PURPOSE: Mutational status of isocitrate dehydrogenase (IDH1) is a defining feature of the World Health Organization classification scheme for high grade gliomas (HGGs). IDH-mutant HGGs confer significantly improved prognoses when compared with IDH-wildtype, which typically describe the most common malignant primary HGGs in adults, namely glioblastoma. HGGs are densely cellular, pleomorphic tumors with high mitotic activity, with glioblastoma having either microvascular proliferation, or necrosis, or both. We hypothesize that integrative analysis of multi-parametric magnetic resonance imaging (mpMRI) via multivariate machine learning (ML), will enhance subtle yet important radiographic HGG characteristics, and reveal imaging signatures determinant of IDH1 mutational status. METHODS: 86 HGG patients were retrospectively identified with available pre-operative clinically-acquired mpMRI data (T1, T1-Gd, T2, T2-FLAIR, DTI, DSC-MRI). Each HGG was delineated into subregions of enhancement, non-enhancement, and peritumoral edema/invasion. 342 quantitative imaging phenomic (QIP) features extracted across subregions from all mpMRI, comprising descriptors of size, morphology, texture, intensity, and biophysical growth modeling. Cross-validated sequential feature selection determined the most discriminative QIP features for our integrative ML predictor of IDH1 status. The predicted classifications, following a 10-fold cross-validation, were compared with the IDH1 status obtained by next generation sequencing, or immunohistochemistry. RESULTS: 61 QIP features, primarily descriptive of tumor texture, were determined as most important for an IDH1 imaging signature. Using this signature, our predictor classified IDH1 mutational status with an accuracy of 88.4% (sensitivity=66.7%, specificity=92.9%). CONCLUSION: Quantitative analysis of clinically-acquired mpMRI reveals subtle/visually-imperceptible, yet informative features, which integrated via ML yield a non-invasive in vivo IDH1 imaging signature in HGG. Knowledge of IDH1 mutational status at initial presentation can influence therapeutic decision-making, which will have a significant impact on patient care. Particularly in this precision medicine era, as mutant-IDH enzyme inhibitors and immunotherapy targeting IDH-mutant tumor cells are developed, imaging to diagnose and follow IDH-mutant tumors can be invaluable. *equal contribution

NIMG-41. NON-INVASIVE DETECTION OF IDH-WILDTYPE GENOTYPE IN GLIOMAS USING DYNAMIC ^{18}F-FET-PET

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PURPOSE: Gliomas with an isocitarate dehydrogenase (IDH) wildtype (wt) status have a dismal prognosis comparable to glioblastoma as reflected by the 2016 WHO classification of gliomas. In order to ensure timely adjustment of surgical and adjuvant treatment strategy, demand for non-invasive imaging methods is high. 18F-FET-PET has been shown to be an important diagnostic tool for glioma management, delivering information on prognosis and therapy response. Aim of this study was to evaluate dynamic 18F-FET-PET for non-invasive evaluation of IDH wt status prior to therapy. METHODS: 341 patients with WHO II-IV glioma were included, in whom IDH mutation status, MRI and dynamic 18F-FET-PET scans were available at initial diagnosis. We assessed sensitivity, specificity, accuracy and positive as well as negative predictive values for maximal tumour-to-background ratio (TBRmax) and minimal time-to-peak (TTPmin) for prediction of IDH wt status in the entire group as well as in the subgroup of non-contrast enhancing (non-CE) tumors. RESULTS: Molecular analyses revealed 178 IDH mutant and 163 IDH wt tumors; 270 patients were classified as FET-positive, in these cases, TTP analysis was performed. Median TBRmax in IDH wt gliomas was 3.1 compared to 2.8 in IDH mutant tumors (p=<0.01). ROC-analyses revealed no reliable cut-off using TBRmax, due to high overlap of the two groups. In contrast, searching for a threshold in dynamic analysis, TTPmin 12.5 minutes identified IDH wt gliomas with a high positive predictive value (accuracy: 79%, PPV: 87%; NPV: 72%). In the subgroup of non-CE glioma in MRI (n=161), IDH wt genotype was identified with an accuracy of 84% (PPV: 83%, NPV: 84 %). CONCLUSION: Dynamic 18F-FET PET using TTPmin analysis provides a reliable non-invasive method for detection of IDH wt genotype especially in tumors without CE on MRI and can help to identify high-risk patients prior to treatment initiation.

NIMG-42. INCIDENCE OF CAVERNOMATOUS LESIONS ON BRAIN MR OF PEDIATRIC PATIENTS TREATED WITH INTRAVENTRICULAR RADIOIMMUNOTHERAPY

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BACKGROUND: Cerebral cavernomas are encountered in pediatric patients with central nervous system (CNS) tumors and may cause neurological compromise. The incidence of cavernomatous lesions on brain MR of pediatric patients treated with intraventricular radioimmunotherapy is unknown. METHODS: 217 patients were treated on institutional clinical trials with 2- 5 serial intraventricular injections 124I- or 131I- (2- 100 mCi) labeled monoclonal antibodies (cRIT) targeting tumor associated antigens. Pre-treatment brain MR at baseline and periodically for follow up over several years were obtained. Brain MR with Gradient echo T2*-weighted imaging and susceptibility-weighted imaging was used to identify cavernomatous lesions. RESULTS: Of 217 patients, 175/ (80.645%) identified as Caucasian, 18 (8.29%) African American, 9 (4.15%) Asian and 15 (6.91%) wished not to identify. Median age at the time of cRIT was 7.6 years (9 months-34.5 years). Median time for MR follow-up examinations was 38.4 months from the time of first cRIT. Cavernomatous lesions were detected in 22/217 (10 %) patients, and in 17/80 patients (21%) who survived > 2 years since cRIT. All in patients with prior external beam radiotherapy 22 (99%) Caucasian, 1 (1%) African American. Diagnoses were, metastatic neuroblastoma (N=12), medulloblastoma (N=8), ependymoma (n=1), pineoblastoma(n=1), and rhabdomyosarcoma (n=1). 12 patients had unifocal cavernomas frontal lobes (N=9), temporal lobe (N=2) or parietal (N=1). Multiple supratentorial and infratentorial lesions were detected in 9 patients; Only 1 patient had lesions involving the brainstem. One patient with cavernomas and concurrent vasculitis had neurologic symptoms; remaining patients were asymptomatic. CONCLUSIONS: Cavernomatous lesions are found in 10% of all patients and 21% of long term survivors treated with cRIT and external beam radiotherapy. The majority of lesions are multifocal and supratentorial. Despite numerous lesions, patients remain without neurologic compromise.

NIMG-43. RADIOLOGICAL CHARACTERISTICS AND NATURAL HISTORY OF ADULT IDH WILD-TYPE ASTROCYTOMAS WITH TERT PROMOTER MUTATIONS

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BACKGROUND: Adult IDH-wildtype astrocytomas with TERT promoter mutations (TERTp) are associated with a poor prognosis. The aim of the present study was to analyze their radiological presentation and natural history. METHODS: We retrospectively reviewed the characteristics of 40 IDH-wildtype TERTp-mutant astrocytomas (grade II n=19, grade III n=21) and compared them to those of 114 IDH-mutant lower grade gliomas (LGG), of 92 IDH-wildtype TERTp-mutant glioblastomas and of 15 IDH-wildtype TERTp-wildtype astrocytomas. RESULTS: Most cases of IDH-wildtype TERTp-mutant astrocytomas occurred in patients aged >50 years (88%) and presented as infiltrative lesions without contrast enhancement (73%) that were localized in the temporal and/or insular lobes (37.5%) or corresponded to a gliomatosis cerebri (43%). Thalamic involvement (33%) and extension to the brainstem (27%) were frequently observed, as was gyriform infiltration (33%). This radiological presentation was different from that of IDH-mutant LGG, IDH-wildtype TERTp-mutant glioblastomas, and IDH-wildtype TERTp-wildtype astrocytomas. Tumor evolution before treatment initiation was assessable in 17 cases. Ten cases demonstrated a rapid growth characterized by the apparition of a ring-like contrast enhancement and/or a median velocity of diametric expansion (VDE) 8 mm/year but 7 cases displayed a slow growth (VDE < 8 mm/year) that could last several years before anaplastic transformation. Median overall survival of IDH-wildtype TERTp-mutant astrocytomas was 27 months. CONCLUSION: IDH-wildtype TERTp-mutant astrocytomas typically present as non-enhancing temporo-insular infiltrative lesions or as gliomatosis cerebri in patients aged >50 years. In the absence of treatment, although rapid tumor growth is frequent, an initial falsely reassuring, slow growth can be observed.

NIMG-44. QUANTITATIVE MULTI-PARAMETRIC IMAGE PROFILING REVEALS REMARKABLE HETEROGENEITY WITHIN IDH-WILDTYPE GLIOBLASTOMA, OFFERING PROGNOSTIC STRATIFICATION BEYOND CURRENT WHO CLASSIFICATIONS

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PURPOSE: The current WHO classifies astrocytomas by IDH mutational status given the significantly poorer prognosis of IDH-wildtype tumors, representing ~95% of de novo glioblastoma. Our previous studies revealed remarkable heterogeneity of these tumors, dividing them in three distinct radiographic subtypes (Rad-S). In this study, we hypothesize that this heterogeneity expands in Rad-S within IDH-wildtype glioblastoma, subdividing them further according to prognosis. METHODS: We analyzed pre-operative multi-parametric magnetic resonance imaging (mpMRI) data (T1,T1-Gd,T2,T2-FLAIR,DTI,DSC) of a retrospective cohort of pathology-proven de novo IDH-wildtype glioblastoma (n=76). Comprehensive quantitative imaging phenomic (QIP) features were extracted from distinct cancerous sub-regions (enhancing, non-enhancing, edematous), using the Cancer Imaging Phenomics Toolkit (CaPTk-www.cbica.upenn.edu/captk). QIP features comprised intensity histogram, volumetric, morphological, statistical, and textural descriptors. Unsupervised clustering of these features alone revealed tumor Rad-S, based on unambiguous clustering assignments across 1000 permutations, that were evaluated through survival and molecular characteristics. RESULTS: Three Rad-S were identified within IDH-wildtype glioblastoma, with statistically significant survival differences (long-:intermediate-:short-survival, median(months)=19.4:12.3:7.0, distribution=19.7%:34.2%:46.1%) measured by Kaplan-Meier analysis (P<0.001,log-rank) and Cox-Model (hazard-ratio=3.21,95%CI:2.51–4.61). Rad-S correlate with survival independent of age, resection-status, post-surgical therapy, additional genetic alterations, and MGMT promoter methylation status. Importantly, long-survival Rad-S, compared to others, showed statistically significant (P<0.001,Kruskal-Wallis) central hypo-intense non-enhancing region surrounded by hyper-intense rim (T1-Gd), lower angiogenesis/neovascularization (DSC) and cell-density (DTI), and higher water concentration (T2). CONCLUSIONS: Quantitative analysis of mpMRI yields three distinct Rad-S within IDH-wildtype glioblastoma offering complementary stratification beyond current WHO classification, which is independent of any factor known to affect prognosis. These Rad-S provide an additional prognostic indicator as a component of precision diagnostics that may impact choice/timing of surgery, chemotherapy, bevacizumab and radiation, allowing personalized treatment. Further, our current understanding in clinical setting is insufficient to explain prognostic differences among the Rad-S. These results provide guidance for ongoing investigation to elucidate pathologic mechanism and consequently targeted therapeutic strategies.

NIMG-45. MULTIVARIATE PATTERN ANALYSIS OF DE NOVO GLIOBLASTOMA PATIENTS OFFERS IN VIVO EVALUATION OF O^6-METHYLGUANINE-DNA-METHYLTRANSFERASE (MGMT) PROMOTER METHYLATION STATUS, COMPENSATING FOR INSUFFICIENT SPECIMEN AND ASSAY FAILURES

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BACKGROUND: The promoter methylation status of the gene encoding for the repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) indicates increased efficacy of current standard of care therapy, which is concomitant adjuvant chemoradiotherapy with temozolomide. The MGMT promoter methylation status (MGMTpms) is typically determined as MGMT-methylated or MGMT-unmethylated by tissue-based polymerase chain reaction assays, which can be limited by inadequate specimen or assay failures. Thus, we investigate the hypothesis that integration of subtle, yet distinctive, quantitative imaging phenomic (QIP) features using machine learning may lead to non-invasive determination of MGMTpms. METHODS: We identified a retrospective cohort of 122 (46 MGMT-methylated) pathology-proven de novo glioblastoma patients with available baseline pre-operative multi-parametric magnetic resonance imaging (mpMRI) data (T1, T1-Gd, T2, T2-FLAIR, DSC, DTI). MGMTpms was obtained through MGMT methylation testing (pyrosequencing across 4 CpG sites in the MGMT promoter). Following delineation of distinct abnormal sub-regions (enhancing, non-enhancing, edematous), comprehensive and diverse QIP features were extracted using the Cancer Imaging Phenomics Toolkit (CaPTk, www.cbica.upenn.edu/captk), capturing intensity, volume, morphology, statistics, and texture of each sub-region. A support vector machine multivariately integrated these features towards a non-invasive marker of MGMTpms. RESULTS: The cross-validated accuracy of our MGMT marker in classifying the mutation status in individual patients was 84.43% (sensitivity=80.43%, specificity=86.84%, area under the curve [AUC]=0.85). Our marker revealed MGMT-methylated tumors with lower neovascularization and cell density, when compared with MGMT-unmethylated tumors, and a distinct spatial distribution pattern between MGMT-methylated and MGMT-unmethylated tumors, with the latter being more lateralized to the right hemisphere. CONCLUSION: Multivariate integrative analysis of QIP features extracted from mpMRI yields an accurate, non-invasive marker of MGMTpms in glioblastoma. The proposed non-invasive MGMT marker may contribute to (i) MGMTpms determination for patients with inadequate tissue/inoperable tumors, (ii) stratification of patients into clinical trials, (iii) patient selection for targeted therapy, and (iv) personalized treatment planning. *equal contribution

NIMG-46. LONGITUDINAL RESTING-STATE FUNCTIONAL CONNECTIVITY CONFIRMS MARIZOMIB (MRZ) CROSSES THE BLOOD BRAIN BARRIER (BBB) AND CORRELATES WITH HALLUCINATION SEVERITY IN RECURRENT GBM PATIENTS

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INTRODUCTION: MRZ is a second-generation, irreversible proteasome inhibitor currently in clinical trials for GBM. MRZ has ability to cross the BBB in animal models. CNS side-effects (including hallucinations and cerebellar ataxia) are both common adverse events. Here we report on the regional fMRI-derived functional connectivity changes associated with hallucination severity (graded using CTCAE 4.03) after MRZ treatment, administered at day 1, 8, and 15 every 28 days. METHODS: Longitudinal resting-state fMRI whole-brain volumes (TR 2500ms, TE 20ms, flip angle = 71°, slice thickness = 3mm, gap = 0 mm, FOV 19.2 cm, matrix = 64 x 64, 51 slices, 120 volumes) were acquired on six participants at baseline (day 0) and days 1 and 15 of the treatment cycle. Preprocessing included: linear detrending, band-pass filtering, EPI signal from the white matter and CSF masks1; hand-drawn tumor masks; rigid-body realignment parameters; and motion and artifact scrubbing2 as implemented in the CONN toolbox3. Linear models were used to assess the correlations of hallucination severity and longitudinal functional connectivity changes in regions from the Harvard-Oxford atlas. RESULTS: After one day of treatment, we found hallucination severity was associated with decreased functional connectivity between the left lingual gyrus and both the left cerebellum (T(4)=-12.78, p<0.03 FDR) and left temporal cortex (T(4)=-9.56, p<0.04 FDR). After fifteen days, the association persisted but became more prominent between the bi-lateral temporal-occipital fusiform cortex and the bi-lateral cerebellum (left: T(4)=-20.20, p<0.005 FDR; right: T(4)=-17.75, p<0.008 FDR) along with decreased local efficiency in the left lateral occipital cortex (T(4)=-11.25, p<0.05 FDR). CONCLUSIONS: Our data suggest that MRZ induce changes in functional connectivity in selected brain areas, including the optic pathways and the cerebellum, confirming MRZ ability to cross the BBB in humans. Research to determine the relation between functional connectivity and response to MRZ are ongoing.

NIMG-47. A HISTOGRAM-BASED, BACK-PROJECTION METHOD FOR TREATMENT RESPONSE ASSESSMENT IN GLIOBLASTOMA USING MULTI B-VALUE ADVANCED DIFFUSION MRI

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INTRODUCTION: Early and accurate assessment of therapeutic response in glioblastoma is important for clinical patient management, and as platform for clinical trials of novel therapies. Current response criteria such as RANO (Response Assessment in Neuro-Oncology) rely on semi-quantitative measurements with limited sensitivity and specificity, especially early during treatment. Functional diffusion maps based on voxel-to-voxel comparison of quantitative diffusion measures are confounded by change in tumour size. Towards improving response assessment, we propose a histogram-based, voxel back projection method using advanced quantitative diffusion MRI. METHODS: We used least-square fitting to model four dif-

fusion parameters from mono-, bi-, and stretch-exponential models, and performed a histogram analysis of all voxels located within ROIs dictated by the radiotherapy-planned clinical target volume. Histograms were generated for 10 patients with GBM at 2 time-points, before, and at 6 weeks of treatment with standard-of-care regimen (RT with concomitant and adjuvant temozolomide). For each parameter, percentile ranges corresponding to diffusion values with the greatest cumulative difference between the time points were identified, and associated voxels were back-projected onto their respective maps for spatial correspondence and comparison with standard radiological imaging. RESULTS & DISCUSSION: The greatest cumulative difference in diffusion parameters measured at baseline and at 6 weeks of treatment, spatially corresponded to voxels lying within regions of high FLAIR signal intensity and regions radiologically-defined as healthy brain. The magnitudes of diffusion imaging parameters within these regions were heterogeneously distributed and did not show a direct spatial correspondence to radiological signal intensities. This heterogeneous spatial distribution may be influenced by microcellular changes, for example, in areas of infiltrative tumor which are not visible on standard imaging. Further assessment of this dataset, augmented by on-going patient recruitment across multiple centres, will provide insight to the prognostic value of advanced diffusion MRI as a method for response assessment in glioblastoma.

NIMG-48. VOLUMETRIC RESPONSE TO TTFIELDS IN NEWLY DIAGNOSED GBM

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INTRODUCTION: Optune TTField treatment is an established, non-invasive therapy for patients with newly diagnosed or recurrent glioblastoma (GBM). Treatment efficacy was evaluated, showing significant improvement of both, overall and progression free survival. For analysis, the MacDonald criteria were used. We performed a 3D volumetric analysis of patients with newly diagnosed GBM who underwent resection and chemoradiation with additional TTFields treatment. RESULTS: Between February 2016 and April 2017, 14 patients were initiated with TTField treatment. For analysis of volumetric response rates, we were able to include 7 patients (newly diagnosed GBM, device usage over 60%, treatment duration over 3 months). Segmentation and volumetric analysis was done for contrast enhancing tumor on T1 imaging (MPRAGE, 1mm slice thickness). The median age was 48 years (range 17–68 years) at the date of prescription. Median preoperative volume was 20.3 cc (range: 1.12–58.6cc), postoperative volume was 6.3 cc (range: 0–14.2cc) and volume upon analysis was 1.2 cc (range: 0–7.1cc). Average device usage (ADU) of these patients was 89.47%. The median change of volume from postoperative to posttreatment volume was 4cc (range: 0–7.1cc) in patients with a median device usage of over 80%; whereas in patients with lesser than 80%, the mean volume change was 2.2cc (range 0–3.2cc). CONCLUSION: TTFields are generally accepted and efficacious for treatment of GBM. Our analysis showed that the combination of radiation, Temozolomide and TTFields lead to a reduction in contrast enhancing tumor volume. The effect seemed to be related to treatment duration.

NIMG-49. ELECTRIC FIELD INTENSITIES DELIVERED BY TUMOR-TREATING FIELDS (TTFIELDS) TO GLIOBLASTOMA REGIONS: EFFECT ON TREATMENT RESPONSE ASSESSED BY AMINO ACID PET

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OBJECTIVE: The main goal of this study was to evaluate if the magnitude of electric field (EF) intensities delivered to glioblastomas by TTFields was related to interval changes of tumoral amino acid uptake measured by PET. METHODS: Ten patients (mean age: 55 years) with residual or recurrent glioblastoma underwent alpha[C-11]-methyl-L-tryptophan (AMT)-PET followed by TTFields treatment. The PET scan was repeated 44–90 days later (mean: 69 days). To simulate delivery of TTFields to the tumor, we used the patient's high-resolution MRI and CT scans to create patient-specific realistic head models comprised of various tissue compartments. Each tissue was assigned appropriate electrical properties. For each direction of treatment (antero-posterior and left-right), two 9-disk transducer arrays were simulated using disks placed according to the patients NovoTAL-planned arrays. To generate TTFields, an alternating voltage difference (200V, 200 KHz) was imposed on the outer surfaces of the disks. The simulations were performed using ZMT's Sim4Life V3.0 electro-quasi-static solver. The field intensities were then normalized to simulate 2A peak-to-peak current supplied by the device. EF maps were fused with the patients' PET images, and mean EF intensities were measured in the PET-defined tumor region at baseline and follow-up and correlated with the interval change of the tumoral AMT uptake ratios. RESULTS: A total of 18 distinct tumor regions were evaluated in the 10 patients. The mean EF intensity delivered to the metabolically active tumor region varied widely (1.24–2.56 V/cm, mean: 1.93 V/cm). Out of 16 tumor regions of 9 patients with good compliance with TTFields, AMT uptake ratios showed an interval decrease in 13. Higher mean EF intensity delivered to the tumors was associated with a more robust decrease of tumoral AMT uptake (r=-0.55, p=0.028). CONCLUSIONS: These data suggest that the magnitude of electric field intensities delivered to metabolically active glioblastoma regions may affect treatment responses.

NIMG-50. GROWTH PATTERN AND PROGNOSTIC FACTORS OF UNTREATED NONFUNCTIONING PITUITARY ADENOMAS

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OBJECTIVE: Pituitary adenomas (PAs) are often detected as incidental findings. However, the natural history remains unclear. The objective of this study was to evaluate the natural history and growth pattern of untreated PAs. METHODS: Between 2003 and 2014, 59 PAs were managed with clinico-radiological follow up for longer than 12 months without any kind of therapeutic intervention. Tumor volumes were calculated at initial and last follow-up visit, and tumor growth during the observation period was determined. Data were analyzed according to clinical and imaging characteristics. RESULTS: The mean initial and last tumor volume and diameter were 1.83 ± 2.97 cc and 13.77 ± 6.45 mm, 2.85 ± 4.47 cc and 15.75 ± 8.08 mm, respectively. The mean annual tumor growth rate was 0.33 ± 0.68 cc/year during a mean observation period of 46.8 ± 32.1 months. Sixteen (27%) PAs showed tumor growth. The initial tumor size (HR1.140, 95% CI 1.003 1.295, p=0.045) was the independent predictive factor that determined the tumor growth. Six patients (11%) of 56 conservatively managed non-symptomatic PAs underwent resection for aggravating visual symptoms with mean interval of 34.5 months from diagnosis. By Cox regression analysis, PAs of last longest diameter over 21.75 mm were a significant prognostic factor for eventual treatment. CONCLUSION: The initial tumor size of PAs was independently associated with the tumor growth. Six patients (11%) of conservatively managed PAs were likely to be treated eventually. PAs of last follow-up longest diameter over 21.75 mm were a significant prognostic factor for treatment. Further studies with a large series is required to determine treatment strategy.

NIMG-51. THE IMPACT OF FUNCTIONAL MAGNETIC RESONANCE IMAGING ON CLINICAL OUTCOMES IN A PROPENSITY-MATCHED LOW GRADE GLIOMA COHORT

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BACKGROUND: This study aims to evaluate the impact of preoperative functional magnetic resonance imaging (fMRI) on clinical outcomes in low grade glioma (LGG) patients. METHODS: In a retrospective propensity-matched cohort study, we compared LGG patients based on whether they underwent fMRI as part of preoperative assessment. Twelve LGG patients who underwent preoperative fMRI were selected, and a contemporaneous group of twelve control LGG patients who did not undergo fMRI were matched to the fMRI group based on age, sex and 1p/19q status. RESULTS: Functional MRI group subjects tended to have more aggressive surgeries (67% resection, 33% biopsy) than the control group (33% resection, 67% biopsy). There were no significant differences in outcomes between the groups. Time between clinical assessment and surgery tended to be longer in the fMRI group (6.3 +/- 4.2 weeks) than in the control group (2.7 +/- 2.2 weeks). Extent of resection was similar between the cohorts. Functional MRI groups subjects had lower preoperative functional status, and tended to have a greater postoperative functional status improvement than control group subjects. Mean survival was not significantly different (fMRI group five year survival 88.9%, control group five year survival 61.1%). CONCLUSIONS: We evaluated the impact of preoperative fMRI in patients with LGG in this propensity-matched cohort study. This study has not demonstrated any significant difference in outcomes between the fMRI and control groups, although there were non-significant trends for patients who underwent fMRI to undergo more aggressive surgical interventions, and have greater postoperative functional status improvement.

NIMG-52. PREDICTION OF SURVIVAL OUTCOME WITH RADIOLOGICAL PHENOTYPES IN IDH-WILD TYPE LOWER GRADE GLIOMAS BASED ON MACHINE LEARNING

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BACKGROUND: IDH-wild type lower grade gliomas are known to be similar to glioblastoma in terms of genetic alterations and prognostically heterogeneous. The purpose of this study was to investigate the prognostic value of imaging phenotypes using machine learning in IDH-wild type lower grade gliomas. METHODS: Preoperative MRIs of 112 patients with histopathologically confirmed IDH-wild type grade II or III gliomas were retrospectively analyzed according to the Visually Accessible Rembrandt Images (VASARI) features set. A radiologic risk score (RRS) for overall survival (OS) and progression free survival (PFS) was produced by selected features and their regression coefficients from LASSO and Elastic net regression model with 100 times of repeated cross validation. Multivariable Cox analysis was performed including age, Karnofsky Performance score (KPS), grade, extent of resection and RRS. The added predictive value of RRS was calculated by comparing C-indices after bootstrapping between multivariable Cox models with and without RRS. RESULTS: For OS and PFS prediction, a Cox regression model comprising clinical features showed C-index of 0.741 and 0.737, respectively. When RRS derived from LASSO (RRS_L) was added to the model, C-index increased to 0.783 and 0.782 for OS and PFS prediction, respectively, without statistical significance. RRS_L was a strong predictor for both OS (HR 3.31) and PFS (HR 3.24). When RRS derived from Elastic net (RRS_E) was added to the model, the model achieved superior performance with C-index being 0.793 and 0.783 for OS and PFS prediction, respectively, with statistical significance ($p < 0.05$). RRS_E was an independent predictor for both OS (HR 2.62) and PFS (HR 2.80). CONCLUSION: RRS derived from MRI features using machine learning was independent predictors for survival in patients with IDH-wild type lower grade gliomas. Radiological phenotypes may have added prognostic value in patients with IDH-wild type lower grade gliomas.

NIMG-53. REPEATABILITY OF O-(2-18F-FLUOROETHYL)-L-TYROSINE POSITRON EMISSION TOMOGRAPHY (FET-PET) SCANNING AND THE INFLUENCE OF PROTEIN INTAKE IN GLIOMA

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BACKGROUND: FET-PET scanning is used in routine clinical management and evaluation of glioma brain tumours. Clinical guidelines recommend fasting 4–6 hours before a FET-PET scan. Animal studies indicate that protein intake prior to the scan does not reduce FET-uptake in tumours, but there are no human studies published. Critical knowledge of test-retest variation of FET-PET imaging of glioma tumour volume as well as uptake and the impact of protein intake is sparse. AIM: This study investigated the repeatability of FET-PET scanning and the impact of protein-intake prior to FET-PET scanning of gliomas. MATERIALS AND METHODS: 20 histologically confirmed glioma patients were included in this prospective study. Subjects were divided into two groups; no protein (NP, n=11) and protein (P, n=9). All subjects underwent two 40 min. dynamic FET-PET scans on a Siemens PET/CT scanner with an interval of maximum 7 days between each scan. Group P consumed 24g of protein (Nutricia Nutridrink Compact®) orally an hour before the second scan. Blood samples were drawn before and after protein intake for determining the plasma-amino-acid concentration. None of the patients were in treatment at the time of the scans. Volumes of interest were delineated on the 20–40 min 18F-FET PET acquisition co-registered to contrast enhanced T1-weighted MRI. The two scans were compared by calculating the absolute change between healthy appearing cortex (B), the maximal and the mean tumour uptake normalized to B (Tmax/B; Tmean/B) and by measuring the biological tumour volume (BTV) defined as uptake above 1.6 mL of mean in healthy appearing brain. RESULTS: The absolute change in FET tumour metrics, mean (range) Tmax/B: NP=0.12[-0.11;0.52] P=0.16[-0.11;0.82] Tmean/B: NP=0.20[-0.06; 2.00] P=0.06[-0.03;0.22] BTV(mL): NP=0.76[-1.50;3.30] P=2.59[-0.02; 12.0] All results were non-significant, p-value > 0.05 CONCLUSION: Tumour day-to-day variation might be greater than first anticipated. Updated results will be presented.

NIMG-54. SPATIAL DISTRIBUTION ATLASES OF POST-TREATMENT MRI SCANS REVEAL DISTINCT HEMISPHERIC DISTRIBUTION OF GLIOBLASTOMA RECURRENCE FROM PSEUDO-PROGRESSION

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PURPOSE: A significant challenge in post-treatment assessment of Glioblastoma is differentiating tumor recurrence (TR) from pseudo-progression (PsP), a radiation-induced treatment effect on routine MRI scans. Previous studies on pre-treatment MRI suggested that aggressive GBM lesions are spatially localized in the right hemisphere; and are associated with poor survival. We thus hypothesize that aggressive TR lesions appearing on post-treatment scans, will likely be more localized in the right hemisphere, as compared to benign PsP. METHODS: 106 post-treatment MRI studies (35 PsP, 71 TR) were collected from 2 institutions. Confirmation for PsP and TR was obtained either from pathologic resection or MRI follow-up using RANO criteria. Scans were registered to T1-weighted brain atlas (MNI152), followed by expert delineation of enhancing lesion on Gd-T1w MRI and peri-lesional hyperintensities on T2/FLAIR. Population atlases quantifying the frequency of occurrence of enhancing lesion and peri-lesional hyperintensities were constructed by averaging voxel intensities across all patients. Analysis of differential involvement (ADIFFI) based on a two-tailed Fisher's exact test was performed to compute significant differences ($p\text{-value}<0.05$) across PsP and TR voxels. Significant clusters were finally mapped to a structural atlas to provide anatomic localization of TR and PsP lesions. RESULTS: ADIFFI results showed TR prominence in the right parietal lobe with 75% occurrence in enhancing lesion and 61% in peri-lesional T2/FLAIR hyperintensities. PsP lesions were prominent in the left hemisphere, with peri-lesional T2/FLAIR hyperintensities having a multi-focal spatial distribution in the temporal lobe, insula, and putamen, and enhancing lesion being localized at the temporal lobe. CONCLUSION: TR tends to be lateralized towards the right parietal lobe. PsP tends to be multi-focally distributed in the left hemisphere. Such spatial localization on MRI could serve as a biomarker for differentiating PsP and TR. This could allow for immediate treatment changes in patients with recurrence, while avoiding unnecessary treatment for pseudo-progression.

NIMG-55. RADIOMICS ANALYSIS FOR DETECTION OF IDH MUTATION OF GLIOMA USING DIFFUSION TENSOR AND KURTOSIS IMAGES

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OBJECTIVE: Creating a machine learning model using diffusion tensor and kurtosis images are effective for malignancy diagnosing of glioma. However, it is unknown whether an IDH mutation, which is the most important mutation in glioma, can be predicted using diffusion tensor and kurtosis images. The purpose of our research was to predict the IDH mutation by using diffusion tensor and kurtosis images based on a machine learning. METHODS: Between August 2014 and July 2017, 38 consecutive patients with suspected glioma and preoperative MRI including diffusion coefficient (ADC), fractional anisotropy (FA), mean kurtosis (MK), were included in the study. All IDH mutations were identified by Sanger sequencing. A volume of interest (VOI) was created manually and applied to T2-weighted images, diffusion-weighted images (DWI), ADC, FA, MK. 476 per imaging sequence and 2856 features were extracted. RESULTS: 39 datasets were obtained. The number of cases for the IDH mutation and wild-type was 21 cases of the IDH1 mutation (3 diffuse astrocytoma, 8 anaplastic astrocytoma, 4 oligodendroglioma, 3 anaplastic oligodendroglioma, 3 glioblastoma, 18 cases of wild-type(2 anaplastic astrocytoma, 16 glioblastoma). An IDH2 mutation was not observed in our cases. Features were selected by using recursive feature elimination (RFE). The most accurate machine learning model was created using the extracted eight features from ADC, Fa, and MK. The best AUC was 0.95 ± 0.02 in a support vector machine (SVM). Discussion: Imaging sequences useful for the IDH1 mutation identification are ADC, MK, FA, similar to malignancy diagnosing of glioma. However, useful features were different. The machine learning models using diffusion images may be useful for identifying the IDH1 mutations.

NIMG-56. PREDICTIVE MARKERS FOR MGMT PROMOTER METHYLATION IN GLIOBLASTOMAS

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BACKGROUND AND PURPOSE: The Promoter methylation status of the O6-methylguanine-DNA methyltransferase (MGMT) gene has been described as one of the most important predictors for chemotherapeutic response and patients survival in glioblastomas (GBs). Therefore, prediction of the MGMT promoter methylation status by imaging would help to preoperatively decide the overall treatment strategy as well as surgical strategy including placement of BCNU wafers. This study aimed to detect imaging parameters to predict MGMT promoter methylation in GBs using a commercially available software. MATERIALS AND METHODS: We investigated 3 imaging features (ring enhancement, location and laterality) and apparent diffusion coefficient (ADC) parameters in 48 newly diagnosed glioblastomas treated at Keio University Hospital in 2006 or later. For ADC, texture analyses were performed. Regions of interest (ROIs) were drawn manually with reference to the relatively higher signal on contrast-enhanced T1-weighted images excluding necrotic and cystic regions. Mean ADC value and ADC histogram parameters including kurtosis, skewness and entropy were compared with MGMT promoter methylation. Each parameter was evaluated if any correlation with MGMT promoter methylation, and the parameters with significant association with the methylation status were correlated with MGMT positive cell ratio in immunohistochemistry. RESULTS: ADC entropy and mean ADC value were significantly associated with MGMT promoter methylation. The combination of ADC entropy and mean ADC value predicted MGMT promoter methylation with PPV of 81.2%, specificity of 88.9%. ADC entropy and mean ADC value were negatively correlated with MGMT positive cell ratio in immunohistochemistry. CONCLUSIONS: This study demonstrated that texture analyses of apparent diffusion coefficient histograms in GBs using a commercially available software were useful for predicting MGMT promoter methylation.

NIMG-57. IMAGING FEATURES OF POLYMORPHOUS LOWGRADE NEUROEPITHELIAL TUMOR OF THE YOUNG (PLNTY)

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INTRODUCTION: Polymorphous lowgrade neuroepithelial tumor of the young (PLNTY) is a recently described epileptogenic tumor. The imaging features of PLNTY are not well characterized, as the largest published series included only 7 patients with available CT or MR imaging. METHODS: IRB-approved retrospective review of patients diagnosed with PLNTY at Mayo Clinic Rochester (MCR) or via the MCR consultative pathology practice between 01/2015 and 03/2018 with available CT and/or MR imaging. RESULTS: Eight patients with PLNTY met inclusion criteria for this study. Median age was 15 years (range 5 - 59 years), and 7 patients (88%) were female. Six tumors (75%) were temporal and 2 (25%) were extratemporal in location. MR imaging was available for review in all patients, and CT imaging was available in 5 patients (63%). Four of five (80%) of cases with CT imaging demonstrated dense tumoral calcification, and two of the three (66%) MR-only cases showed focal hypointensity on GRE sequences, likely reflecting calcification. Overall, 6/8 (75%) of cases demonstrated definite or probable calcification on imaging. Tumors were generally heterogeneous on FLAIR and T2-weighted images with hyperintense tumor tissue outside the region of calcification. T1-weighted images were likewise heterogeneous, with 2/8 (25%) of tumors demonstrating enhancement on post-gadolinium imaging. DISCUSSION: In this imaging series, the largest reported for patients with PLNTY, dense tumoral calcification and temporal lobe localization were common features, while enhancement was relatively rare. The high rate of tumoral calcification is distinct from other epileptogenic tumors seen in young patients, and may be a useful marker for pre-operative diagnosis.

NIMG-59. VALIDATION OF QUANTITATIVE VESSEL SIZE IMAGING (VSI) IN HUMAN GLIOMAS USING IMAGE-GUIDED STEREOTACTIC BIOPSIES

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OBJECTIVE: Angiogenesis is critical for brain tumor development and malignant transformation1, influencing both prognosis and response to therapy. The vessel size imaging (VSI) 2 is a quantitative technique that estimates the mean diameter of vessels by using multi-echo spin-and-gradient-echo (SAGE) dynamic susceptibility contrast (DSC) perfusion MRI. In the current study, we compared rCBV and VSI maps to histology-equivalent estimates of vessel density and vessel diameter in high grade glioma patients, using stereotactic image-guided biopsies. METHODS: A total of 26 image-guided biopsies were obtained in 11 glioma patients (7 grade III and 4 glioblastoma). MRI examinations were acquired prior to surgery on a 3T MRI, including multi-echo SAGE DSC perfusion (2x gradient echo, 1x asymmetric echo, and 1x spin-echo). One to three biopsy targets (5mm radius) were defined and stereotactically biopsied. Relative cerebral blood volume (rCBV) was calculated3 using DSC data from a single gradient echo. VSI was quantified as VSIm= 0.867(ADC.rCBV)^(1/2)((R_2^*)/R_2^(3/2)) where ADC is the apparent diffusion coefficient (mm2/s), R2* and R2 are transverse relaxation rates. Histologically estimates of VSIHisto. were obtained as previously modelized.4 Results: Our results suggest that no differences exist in vessel density and VSI from histology between grade III and IV. Indeed, ADC, rCBV and VSI are similar within targets for those grades. We observed a significant correlation between rCBV and vessel density (r=0.42, p=0.032) but not between rCBV and VSIHisto.. Interestingly, VSIMRI was independent of vessel density but highly correlated with VSIHisto. CONCLUSION: MR measures of VSI exhibit a strong relationship to histological measures of vessel diameter in high grade glioma and that independently of blood volume and vessel density. References: 1 Digernes et al. JCBFM 2017. DOI: 10.1177/0271678X17694187 2 Kiselev et al. MRM 2005. DOI: 10.1002/mrm.20383 3 Leu et al. JMRI 2016. DOI: 10.1002/jmri.25227 4 Tropres et al. MRI 2004 DOI: 10.1002/mrm.20017

NIMG-60. AUTOMATIC DETECTION OF HIGH AMINO ACID UPTAKE REGIONS IN GLIOBLASTOMA FROM MULTI-MODAL MRI: A FULL 3D U-NET STUDY OF DEEPLY LEARNED PET DATA

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BACKGROUND: Previous studies reported that high amino acid uptake by alpha-[C-11]-methyl-L-tryptophan (AMT)-PET can accurately detect glioblastoma cell infiltration both in enhancing and non-enhancing tumor portions. Thus, AMT uptake is a strong and independent imaging marker of the metabolically active tumor region. However, amino acid PET is not widely available for clinical usage. Here we introduce a novel end-to-end deep learning framework to detect the high tryptophan uptake glioblastoma regions using clinical multi-modal MRI. METHODS: Contrast-enhanced T1, non-contrast T2/FLAIR MR images, apparent diffusion coefficient maps from diffusion weighted imaging, and AMT-PET images were analyzed in 12 patients with glioblastoma (mean age: 57 years). Multi-modal images were spatially co-registered and resampled at the same resolution (1mmx1mmx1mm). A binary mask of the metabolically active tumor was obtained as the ground truth from AMT-PET by applying a previously established threshold of 1.65 tumor/normal cortex ratio. A 3D U-net (Ronneberger et al., 2015; https://arxiv.org/pdf/1505.04597.pdf) was implemented to learn input: multi-modal MRI and output: ground truth using Google TensorFlow library with 4 layers of the encoding and decoding paths. Dice similarity coefficient (DSC) was used as a measure of detectability and also a loss function that was back-propagated through the U-net. RESULTS: Data augmentation was performed to generate 1200 study data by applying random affine transformation to the original data of 12 patients (70%/30% for training/validation set). After 1200 iterations, all DSC values reached 0.98/0.96 in the training/validation set. At the voxel level, the resulting model led to 0.87 ± 0.05 sensitivity, 0.99 ± 0.0 specificity, 0.86 ± 0.06 positive predictive value and 0.99 ± 0.0 negative predictive value. CONCLUSIONS: This study translates the advanced deep learning technique to clinical practice where AMT-PET is currently unavailable. Systematic investigation of the proposed U-net may improve presurgical evaluation in glioblastoma by supplementing conventional multi-modal MRI to approximate glioblastoma volume with high amino acid uptake.

NIMG-61. MANAGEMENT OF INTRAOPERATIVE MRI AND NEURONAVIGATION SYSTEM WITH PET FOR MALIGNANT GLIOMAS MANAGEMENT OF INTRAOPERATIVE MRI AND NEURONAVIGATION SYSTEM WITH PET FOR MALIGNANT GLIOMAS

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OBJECTIVE: Image-guided surgery, such as neuronavigation systems with magnetic resonance imaging (MRI) have become standard techniques for glioma surgeries. However, MRI is limited by correct metabolism of gli-

oma cells. Positron-emission tomography (PET) uses radiotracers to achieve metabolic and molecular imaging, but can only be evaluated preoperatively. We compared the utility of intraoperative real-time MRI (IoMRI) and multiple PET studies (methionine [MET], fluorothymidine [FLT], and fluoromisonidazole [FMISO]) for malignant glioma surgery. METHODS: Between January 2016 and May 2018, 39 patients with gliomas underwent IoMRI, whereas 19 (excluding biopsy cases) underwent multiple PET studies for tumor removal. Of these 19 patients, 12 had recurrence and 7 did not. Excision rate and residual region by IoMRI and PET studies were compared. RESULTS: The respective excision rates in the recurrent and nonrecurrent groups were 91.9% (80.499) and 97.7% (92.7100) with IoMRI, 92.3% (79.297.1) and 99.4% (98.3100) with MET, 94.5% (84.597.5) and 99.2% (97.5100) with FLT, and 95.9% (90.298.3) and 98.7% (93.5100) with FMISO. In the recurrent group, the residual volume of each PET tracer was significantly larger than that of IoMRI. All recurrences wherein accumulation areas of each PET tracer remained in the IoMRI excision areas were detected. Recurrence was observed from these sites, particularly the residual region overlapping residual accumulations of MET and FLT. CONCLUSIONS: Fusion of IoMRI images with preoperative multiple PET images during navigation remains challenging. When accumulation areas of more than two PET tracers, particularly MET and FLT, overlap with the residual IoMRI region, considering tumor removal becomes important.

NIMG-63. ADVANCED IMAGING FOR ASSESSING VOLUMETRIC RESPONSES IN BRAIN METASTASES TREATED WITH CHECKPOINT BLOCKADE

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BACKGROUND: Immunotherapy has been effective therapy for brain metastases (BM) from melanoma and lung cancer, prompting interest in using PD-1 targeting drugs in more patients with BM. However, accurately assessing response in patients undergoing immunotherapy continues to be a challenge. Thus, we prospectively evaluated radiographic characteristics and changes during immunotherapy. METHODS: As part of an ongoing Phase 2 study of pembrolizumab for patients with untreated or progressive, previously treated BM from any histology, patients underwent advanced MRI that includes tumor volume measurements and perfusion imaging with dynamic susceptibility contrast MRI.. To calculate volumetric radiographic response, all enhancing voxels were summated. A volumetric increase of >40% was categorized as progressive disease (PD), a decrease of >60% as partial response (PR), and stable disease (SD) as between -60% and +40%. RESULTS: Forty-eight patients have been enrolled of whom 38 have undergone at least baseline advanced MR imaging. Histologies include 16 with breast cancer (12 HER2-, 4 HER2+), 5 with non-small cell lung cancer, 4 with melanoma, and 11 with other cancers. At baseline, the total number of BM was 1–50+ per patient. Based on summing the entire enhancing intracranial disease burden, best volumetric responses for the 25 evaluable patients include 2 PR, 9 SD, and 14 PD. Cerebral blood volume tended to increase with increasing tumor size. Correlation of volumetric response to patient outcome and standardized response criteria (iRANO) is ongoing. CONCLUSIONS: Pembrolizumab may have activity in metastatic brain cancer. Ongoing analyses are evaluating if physiological MRI can shed light on the biological impact of pembrolizumab and response mechanisms in this patient population.

NIMG-64. A CLINICAL RULE FOR PREOPERATIVE PREDICTION OF BRAF MUTATION STATUS IN CRANIOPHARYNGIOMAS

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Papillary craniopharyngiomas are characterized by BRAF V600E mutations. Targeted therapy can elicit a dramatic radiographic regression of these tumors. Therefore, prediction of BRAF mutation status before definitive surgery could enable neoadjuvant treatment strategies. The aim of this study was to establish preoperative prediction criteria to identify patients with a BRAF mutant craniopharyngioma. Sixty-four patients with craniopharyngioma were included in this study. We determined BRAF mutation status by targeted sequencing. After scoring inter-observer variability between presurgical clinical data and radiographic features, we established a diagnostic rule for BRAF mutation in our discovery cohort. We then validated the rule in an independent cohort. The BRAF V600E mutation was detected in 12 of 42 patients in the discovery cohort. There were no patients under age 18 with BRAF mutation. Calcification was rare in tumors with BRAF mutation ($P < .001$), and 92% of them were supradiaphragmatic in location. Combining these three features older than 18 years, absence of calcification, and supradiaphragmatic tumor location we established a rule for predicting BRAF mutation. In cases where all three criteria were fulfilled, the sensitivity and specificity for the presence of BRAF mutation was 83% and 93%, respectively. In the validation cohort (n=22), the sensitivity was 100% and specificity was 89%. We propose predictive criteria for a BRAF mutation in craniopharyngioma using preoperative clinical and radiographic data. This rule may be useful in identifying patients who could potentially benefit from neoadjuvant BRAF V600E targeted systemic therapies.

NIMG-65. VOLUMETRIC ASSESSMENT OF PERITUMORAL EDEMA: EXCELLENT TOOL FOR DIFFERENTIAL DIAGNOSIS OF CEREBRAL GLIOMAS AND SOLITARY METASTASES

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INTRODUCTION: Differentiating cerebral gliomas from metastatic brain tumors is essential since their management and prognosis is widely different. However it is still challenging to differentiate them without pathologic confirm. In this study, we evaluated the value of tumor volume and peritumoral edema as a tool for differentiating cerebral gliomas and solitary metastases. METHOD: We retrospectively reviewed conventional MR images of 91 patients with supratentorial solitary intra-axial tumor which were pathologically confirmed by surgical procedures. Patients were classified as cerebral glioma (n=57) and metastatic tumor group (n=34). Glioma group was also subdivided as glioblastoma multiform (GBM) and non-GBM subgroups. Tumor volume, peritumoral edema volume and edema index (peritumoral edema volume/tumor volume) were analyzed by using a semi-automated 3D slicer, and compared between groups. RESULT: Cerebral glioma group showed significantly small edema volume (median 48.90ml, interquartile range[IQR] 62.12 versus 94.02ml, IQR 81.85; $p<0.001$) and edema index (2.01, IQR 3.15 versus 5.35, IQR 7.99; $p<0.001$) compared to metastatic tumor group. GBM subgroup also presented large tumor volume (median 31.45ml, IQR 40.29 versus 17.37ml, IQR 21.80; $p=0.007$), small edema volume (52.43ml, IQR 55.06; $p=0.004$) and edema index (2.03, IQR 2.16; $p<0.001$) than metastatic tumor group. There were no difference between non-GBM and GBM subgroups. CONCLUSION: Tumor volume, peritumoral edema volume and edema index can contribute differentiating cerebral gliomas from metastases. In particular, measuring edema index can be an excellent tool for differential diagnosis in addition to other radiologic features.

NIMG-66. COMPARISON OF STEADY STATE AND DYNAMIC BRAIN METABOLISM BY USING 1H MRSI AND HYPERPOLARIZED [1-^{13}C]PYRUVATE IMAGING IN PATIENTS WITH GLIOMA

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Proton magnetic resonance spectroscopic imaging (1H MRSI) is a powerful noninvasive method for assessing the spatial extent and properties of abnormal metabolism in patients with glioma. Hyperpolarized 13C metabolic imaging is a new molecular imaging modality that can be used to assess real-time changes in metabolism and has been shown to be safe and feasible in patients with glioma. In this study, both 3D 1H lactate-edited MRSI and hyperpolarized [1-13C]pyruvate imaging data were obtained and compared in thirteen patients with glioma to assess steady state versus dynamic brain metabolism. FLAIR images from the 1H examination were registered to the FSE images from the 13C examination for each subject. The correspond-

ing transformation matrix was then applied to spectra and metabolite maps from the excitation volume of the 1H MRSI data. The volumes of the anatomic and metabolic lesions varied between patients, and using a multi-slice frequency specific EPI sequence allowed improved coverage of the lesion of interest compared to a 2D dynamic EPSI sequence. The choline-to-NAA index values and levels of steady state lactate peak heights from the 1H MRSI data were elevated in the lesion, while 13C bicarbonate levels were reduced and 13C lactate levels were similar or lower compared with normal appearing brain. The initial results indicated that there was a negative association between estimates of hyperpolarized 13C lactate/pyruvate and steady state normalized lactate peak heights, and a positive association between hyperpolarized 13C lactate/pyruvate and Cho/NAA in the T2 lesion. For one patient who received repeated examinations within 2 months and was assessed as having stable disease after treatment, the 13C lactate-to-pyruvate ratio in the T2 lesion was 0.65 vs. 0.35 and 0.43 vs. 0.43 in normal appearing brain. Future studies will evaluate a larger patient population to see whether these relationships hold up.

NIMG-67. CLINICAL APPLICATIONS OF QUANTITATIVE THREE-DIMENSIONAL MRI ANALYSIS FOR PEDIATRIC EMBRYONAL BRAIN TUMORS

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OBJECTIVES: Pediatric embryonal brain tumors include medulloblastoma, supratentorial primitive neuroectodermal tumor, and pineoblastoma. The purpose of this study was to investigate the prognostic utility of quantitative three-dimensional (3D) magnetic resonance imaging (MRI) radiomic analysis for primary pediatric embryonal brain tumors. METHODS: Thirty-four pediatric embryonal brain tumor patients with concurrent pre-operative T1-weighted post contrast (T1PG) and T2-weighted fluid-attenuated inversion recovery (FLAIR) MR images were identified from an institutional database. The median imaging follow-up was 5.2 years. Radiomic features were extracted from axial T1PG and FLAIR contours using MATLAB, and 15 features were selected for analysis based on qualitative radiographic features with known prognostic significance for pediatric embryonal brain tumors. Logistic regression, linear regression, receiver operating characteristic curve, Harrells C index and Somers D index were used to test the relationships between radiomic features, demographic variables and clinical outcomes. RESULTS: We found that pediatric embryonal brain tumors in older patients had increased normalized mean tumor intensity (P=0.05, T1PG), decreased tumor volume (P=0.02, T1PG) and increased markers of heterogeneity (P0.01, T1PG and FLAIR) relative to younger patients. We identified 10 quantitative radiomic features that delineated between medulloblastoma, pineoblastoma and supratentorial primitive neuroectodermal tumor, including size and heterogeneity (P0.05, T1PG and FLAIR). Decreased markers of tumor heterogeneity were predictive of neuraxis metastases and trended towards significance (P=0.1, FLAIR). Tumors with increased size (AUC=0.7, FLAIR) and decreased heterogeneity (AUC=0.7, FLAIR) at diagnosis were more likely to recur. CONCLUSIONS: Quantitative radiomic features are associated with pediatric embryonal brain tumor patient age, histology, neuraxis metastases and recurrence, and could be used for risk stratification.

NIMG-68. MRI CHANGES IN NEWLY DIAGNOSED GLIOBLASTOMA PATIENTS TREATED AS PART OF A PHASE II TRIAL WITH BAVITUXIMAB, RADIATION, AND TEMOZOLOMIDE

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BACKGROUND: Glioblastoma and tumor endothelial cells express phosphatidylserine, a highly immunosuppressive membrane phospholipid. Bavituximab a chimeric monoclonal antibody binds to 2-glycoprotein 1 (2-GP1) to form a complex of 2-GP1 with phosphatidylserine, resulting in immune activation against tumor cells and anti-angiogenic effects. Phase I/II trials in other solid cancers have demonstrated response rates up to 85% when bavituximab was given with cytotoxic chemotherapy. Pre-clinical data in glioblastoma models suggested synergistic effects of phosphatidylserine blockade, radiation, and temozolomide (TMZ). METHODS: In this ongoing phase II trial (NCT03139916), adult patients with IDH-wild-type newly diagnosed glioblastoma receive 6 weeks of chemoradiation, followed by 6 cycles of adjuvant TMZ (C1-C6 aTMZ). Bavituximab (3 mg/kg) is given weekly, starting week 1 of chemoradiation, for 18 weeks with the option to continue if tolerated. Physiologic MRIs are performed pre-treatment, pre-C1, pre-C3, and pre-C5 aTMZ. Within the enhancing tumor region, we measured median tumor Ktrans (reflecting vascular permeability) and relative cerebral blood volume (rCBV). Median percent changes during treatment were compared to pre-treatment values. RESULTS: To date, 25 of 36 anticipated patients have enrolled (10 with MGMT promoter methylation). All patients underwent pre-treatment scans. 13 have evaluable pre-C1 and 8 pre-C3 aTMZ scans. On the pre-C1 MRIs, enhancing volume decreased by 39% and median tumor Ktrans and rCBV did not change significantly. On the pre-C3 MRIs, enhancing volume decreased by 11% and Ktrans and rCBV decreased by 17% and 21%, respectively. Five patients experienced radiographic disease progression after a median of 2.6 months and 1 patient died 76 days after diagnosis due to disease progression. Bavituximab was generally well tolerated. CONCLUSIONS: Combining bavituximab with radiation and temozolomide results in decreased enhancing tumor volume, permeability, and cerebral perfusion. Continued patient accrual and imaging marker evaluation are underway to investigate the correlation between bavituximab, MRI changes, and survival.

NIMG-69. CHANGES IN SIGNAL INTENSITY RATIOS OF GLOBUS PALLIDUS AND DENTATE NUCLEUS ON UNENHANCED T1-WEIGHTED IMAGES AFTER MULTIPLE ADMINISTRATIONS OF MACROCYCLIC GADOLINIUM-BASED CONTRAST AGENTS IN BRAIN TUMOR PATIENTS

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INTRODUCTION: Gadolinium-based contrast agents (GBCAs) are routinely used in MRI studies performed for evaluation of brain tumors. Recent studies have demonstrated, progressively increased signal intensity in the globus pallidus (GP) and dentate nucleus (DN) on T1-weighted brain images obtained in patients undergoing repeated MR studies. Linear GBCAs result in more retention and longer time of retention than macrocyclic GBCAs. The aim of this study is to evaluate changes in signal intensity ratios of the GP and the DN on unenhanced T1-weighted images after multiple administrations of macrocyclic GBCAs in brain tumor patients. METHODS: The study included 37 patients with primary brain tumors who had more than 3 MRI evaluations (range: 4–10 studies). Ten of them (27%) have not receive radiation therapy. Patients were divided into two groups: Group 1 included 24 patients who had Gadoterate meglumine (Dotarem®) enhanced MRI and group 2 included 13 patients who had Gadobutrol (Gadavist®) enhanced MRI. Median age of group 1 patients was 46 (24–69) years and of group 2 it was 56 (32–67). Two radiologists conducted a quantitative analysis of unenhanced T1-weighted images by using region of interest measurements. The difference in mean GP-to-thalamus (GP\TH) signal intensity ratio, DN-to-middle cerebellar peduncle (DN\MCP) signal intensity ratio between the first and last examinations for each patient were calculated. RESULTS: In both groups GP \TH Ratio difference was greater than 0 (Gadavist 0.08 ± 0.06, Dotarem 0.02 ± 0.08 [P=0.035]) following multiple MRI examinations. There was no significant change in DN\MCP ratio in both groups (Gadavist 0.02 ± 0.07, Dotarem -0.01 ± 0.08 [P=0.230]). CONCLUSION: A significant increased GP\Thal ratio is associated with multiple administrations of both macrocyclic agents, but to a greater extent with administration of Gadavist possibly reflecting the difference in elimination and stability of both contrast agents. Further studies are needed to identify clinical implications, if any, of these findings.

NIMG-70. QUANTITATIVE IMAGE ANALYSIS AND MACHINE LEARNING TECHNIQUES FOR DISTINGUISHING TRUE PROGRESSION FROM PSEUDOPROGRESSION IN PATIENTS WITH GLIOBLASTOMA

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PURPOSE: Imaging follow up of glioblastoma patients, after maximal safe resection and radiochemotherapy, commonly demonstrates new/increasing enhancement around the tumor bed concerning for tumor progression/recurrence (TP). However, in ~50%, this enhancement represents primarily treatment-related changes including pseudoprogression (PsP)

within six months from completion of chemoradiation. We seek non-invasive imaging biomarkers, reproducible via publicly-available software, capable of distinguishing TP from PsP. We hypothesize that quantitative analysis of extensive, complementary features extracted from multi-parametric magnetic resonance imaging (mpMRI), via advanced machine learning (ML) techniques, can yield such robust imaging biomarkers. METHODS: We evaluated independent discovery (n=40) and replication (n=23) cohorts of glioblastoma patients, with available mpMRI (T1, T1-Gd, T2, T2-FLAIR, DTI, DSC) and who underwent second resection due to possible recurrence. Extensive features from the mpMRI scans were extracted and quantitatively analyzed via a dynamically-growing software platform specialized in radiographic analysis (cancer imaging phenomics toolkit, or CaPTk - www.cbica.upenn.edu/captk) to derive phenotypic imaging signatures of TP and PsP. Principal components analysis of intensity distributions, morphological, statistical, and texture descriptors, were integrated via support vector machines to evaluate the imaging profile of the target tissue and distinguish among i)TP, ii)mixed response, and iii)PsP. Independently, board-certified neuropathologists evaluated the resected tissue by blindly classifying it in the above three categories based on geographic necrosis, dystrophic calcification, vascular changes, mitotic figures, pseudopalisading necrosis, and Ki67. RESULTS: Tissue classified as TP by the neuropathologists revealed higher angiogenesis based on DSC-derived features; higher cellularity based on DTI-derived features; and lower water concentration (T2, T2-FLAIR). Our quantitative analysis detected TP with 83% accuracy (sensitivity:92% ,specificity:73%,AUC:0.83) and PsP also with 83% accuracy (sensitivity:79%,specificity:100%,AUC:0.90). CONCLUSION: Advanced quantitative mpMRI analysis and ML reveals non-invasive *in vivo* markers of TP versus PsP of glioblastoma that are directly associated with pathological changes. Integration of the proposed method on CaPTk facilitates its potential widespread use.

NIMG-72. A NOVEL ARRAY LAYOUT FOR DELIVERING TTFIELDS TO THE WHOLE BRAIN

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INTRODUCTION: Tumor Treating Fields (TTFields) is an antimitotic cancer treatment approved for the treatment of Glioblastoma Multiforme. TTFields are delivered using 2 pairs of transducer arrays. When treating Glioblastoma, the array positioning on the head is planned to maximize TTFields dose delivered to the tumor. This results in an increased dose of TTFields at the tumor, whilst reducing the intensity of the field in other regions. In some clinical scenarios, such as treatment of multiple brain metastases, it might be desirable to deliver TTFields at therapeutic intensities to the entire brain, thereby ensuring that even microscopic lesions receive a therapeutic dose of TTFields. Here we present novel transducer array layouts designed to deliver a uniform distribution of TTFields to the entire brain. Methods Computer simulations were used to calculate the field distributions generated by different array layouts. The simulations utilized a realistic computerized head model of a 40+ years old human mal. Delivery of TTFields using pairs of array layouts placed at different locations on the head and neck was simulated using Sim4Life V3.0 (ZMT Zurich). To analyze the field distributions, the brain was divided into five regions: 1) the cerebellum, brain stem and other infra-tentorial anatomical regions; 2–5) four quadrant of the cerebrum. The mean and median field intensities in the five regions generated by each layout were calculated and compared. Results A layout in which one pair of arrays is placed on the right temple, and left scapula and the second pair placed on the left temple and right scapula yielded a uniform intensity distribution within the brain. (median intensities between 1.5 V/cm to 1.7 V/cm within all regions). Conclusion We have identified a novel TTFields array layout that will be beneficial in clinical scenarios where treatment of the entire brain with TTFields is desired.

NIMG-73. RADIOMICS OF GLIOBLASTOMA FOR PREDICTING MGMT PROMOTOR METHYLATION STATUS AND PROGNOSIS

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O-6-methylguanine-DNA methyltransferase promotor methylation (pMGMT-met) is identified as a favorable prognostic factor for glioblastoma (GBM) and patients with GBM containing pMGMT-met benefit from temozolomide. On the other hand, radiomic analysis that rely on quantitative texture feature of radiological images has rapidly emerged in the field of neuro-oncology. In this study, the authors attempted to build a radiomic-based prediction model for pMGMT-met status of GB. Building a prognostic model was further attempted by radiomic analysis of conventional MRI. Preoperative MRI (non-enhanced and enhanced T1WI, T2WI, and FLAIR) from 207 newly diagnosed GBM (nGMB) patients were included in this study. Total of 489 texture features including first order feature (histogram of pixels), second order feature (Gray level co-occurrence matrix and Grey level run length matrix) from 166 date sets and location data from 187 data set were collected. Predictive modeling for pMGMT-met status was performed based on LASSO regression with 10-fold cross-validation which was repeated 5 times. Supervised component principal analysis was used for prognosis prediction. One hundred and four cases were pMGMT-met and 103 patients were MGMT promotor unmethylated (pMGMT-unmet). Predictive accuracy of pMGMT-met status was 68% modeled by 28 significant radiomic features. Furthermore, 20 radiomic features correlated with prognosis the analyzed cohort was categorized into high risk and low risk group by use of radiomic score (P value <0.0069). Radiomic score and pMGMT status were independent prognostic factors. Finally, significant survival difference was observed between radiomic high risk and pMGMT-unmet group, intermediated group (which group consists of radiomic low risk and pMGMT-unmet or radiomic high risk and pMGMT-met), and radiomic low risk and pMGMT-met group (P value <0.0001). In conclusion, the current study revealed that biological characteristics of GBM is embedded in radiomic features, recovery of which information could be beneficial for GBM patient care.

NIMG-75. WHO 2016 GRADE II GLIOMA MOLECULAR SUBTYPES HAVE A DISTINCT SPATIAL DISTRIBUTION PATTERN

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INTRODUCTION: Several studies reported a correlation between the anatomic location of gliomas and the genetic background of the tumor. As such, tumor location may contribute to pre-surgical clinical decision-making. Current evidence is mainly derived from smaller series and report single genes or copy numbers, but not the integrated WHO 2016 classification. Our purpose was to assess the location distribution of different WHO 2016 glioma subgroups in a consecutive series of molecularly defined grade II glioma and to create location heatmaps of the low grade glioma molecular subtypes. METHODS: 205 adult patients with a grade II supratentorial glioma diagnosed between 2003–2016 were included in this study (100 *IDH* mutated astrocytoma, 85 *IDH* mutated and 1p19q co-deleted oligodendroglioma, and 20 *IDH* wildtype astrocytoma). All patients were classified according to the WHO 2016 criteria using a dedicated Next-Generation-Sequencing panel. Tumor volume and location were assessed with semi-automatic software on T2-weighted images. All volumes of interest were mapped to a standard brain using affine registration. Location heatmaps were created for each WHO 2016 glioma subgroup by overlaying segmentations on a standard brain. RESULTS: Our location maps confirmed earlier observations that different molecular subtypes of grade II glioma have a different spatial distribution: most *IDH* mutated 1p19q co-deleted oligodendrogliomas were located in the frontal lobes and cortex, while *IDH* mutated astrocytomas were more frequently located in the frontotemporal and insular region. *IDH* wildtype astrocytomas were predominantly located in the basal ganglia and temporal lobes. Our observations were significant as confirmed with voxel-based Fisher's exact tests (figures will be shown at SNO). CONCLUSION: Each WHO 2016 glioma subgroup displayed a distinct pattern of spatial distribution. Our observations may contribute to pre-surgical clinical decision-making and to radiogenomics.

NIMG-76. POST-GADOLINIUM 3-DIMENSIONAL SPATIAL, SURFACE, AND STRUCTURAL CHARACTERISTICS OF GLIOBLASTOMAS DIFFERENTIATE PSEUDOPROGRESSION FROM TRUE TUMOR PROGRESSION

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PURPOSE: Pseudoprogression is often indistinguishable from true tumor progression on conventional 2-dimensional (2D) MRI in glioblastoma multiforme (GBM) patients. The aim of this study was to determine the association between post-gadolinium 3-dimensional (3D) characteristics and clinical state in GBM patients. METHODS: Standardized 3D brain MRI studies were performed, and contrast enhancing portions of each tumor were segmented and analyzed blinded to clinical state using principal component analysis (PCA), medial axis transformation (MAT), and coverage analysis. Associations between the 3D characteristics of the post-gadolinium enhanced regions and the clinical status of patients were performed. RESULTS: A total of 15 GBM patients (male: 11 (73%); median age (range): 62 years (36–72 years)) with a median disease duration of 6 months (range: 2–24 months) were studied cross-sectionally with 6 (40%) patients identified with tumor progression. Post-gadolinium features corresponding to the group with progressive disease exhibited a more spherical and symmetric shape relative to their stable counterparts ($p<0.005$). The predictive value of a more uniformly full post-gadolinium enhanced shell to clinical progression was determined with a sensitivity of 66.7% (95% CI 29.9–92.5), specificity of 100% (54.1–100) and PPV of 100% (p=0.028, 2 tailed Fisher's exact test). There did not appear to be an association between the thickness of the contrast enhanced shell to clinical state. CONCLUSIONS: The application of 3D technology to MRI studies with post-gadolinium imaging data may inform healthcare providers of GBM patients with new insights into pseudoprogression versus true tumor progression based on 3D spatial, surface, and structural patterns of enhancing lesions.

NIMG-77. MULTIMODAL IMAGING OF GLIOBLASTOMA SUBREGIONS: IMPACT ON OVERALL SURVIVAL

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BACKGROUND: Glioblastomas are heterogeneous brain-infiltrating tumors with diverse histopathological and molecular genetic features. Although treatment is mostly focused on the contrast-enhancing tumor mass, combination of advanced structural and molecular imaging can enhance more accurate tumor delineation and capture glioblastoma heterogeneity. Here we combined conventional MRI with diffusion-weighted imaging (DWI) and amino acid PET characteristics to explore imaging-defined glioblastoma subregions and evaluate their potential prognostic value. METHODS: Contrast-enhanced T1, T2/FLAIR MR images, apparent diffusion coefficient (ADC) maps from DWI, and alpha-[^{11}C]-methyl-L-tryptophan (AMT)-PET images were analyzed in 30 patients (mean age: 59 years) with newly-diagnosed glioblastoma. Tumor subregions were identified based on a combination of MRI contrast enhancement, T2/FLAIR signal abnormalities, and AMT uptake on PET. ADC and AMT uptake tumor/contralateral normal cortex (T/N) ratios in these tumor subregions were measured, and their prognostic value for overall survival was determined. RESULTS: A total of 115 MRI/PET-defined subregions were analyzed in the 30 glioblastomas. Most tumors showed not only a high AMT uptake (T/N ratio >1.65, N=27) but also a low-uptake subregion (N=21) within the contrast-enhancing tumor mass. High AMT uptake extended beyond contrast enhancement in 25 cases and correlated with low ADC (r=-0.40, p=0.05), consistent with high cellularity in tumor-infiltrated brain. Non-enhancing T2/FLAIR abnormal subregions had low AMT uptake and high ADC, consistent with vasogenic edema (N=24) or central necrosis (N=18). Cox regression analysis showed that high AMT uptake in the contrast-enhancing tumor subregions was prognostic for overall survival (HR: 7.83 [95%CI: 1.98–31.02], p=0.003), independent of several clinical and molecular genetic prognostic variables. CONCLUSIONS: Amino acid uptake by PET can differentiate metabolically active glioblastoma subregions, often showing dense cellularity, vs. necrotic/edematous areas, both in enhancing and non-enhancing tumor portions. High tryptophan uptake in MRI contrast-enhancing tumor subregions is a strong, independent imaging marker for longer survival in patients with newly-diagnosed glioblastomas.

NIMG-78. EVALUATION OF ISCHEMIC COMPLICATIONS IN REMOVAL OF GLIAL TUMOR

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INTRODUCTION: Recently, the use of navigation system, brain mapping, and intraoperative photo diagnosis during tumor resection becomes possible to prevent maximally neurological function and undergo surgery safety. However, postoperative ischemic complication is unpredictability, as a result, it may cause neurological deficits. We examined retrospectively how predicted ischemic complication and intraoperative important point to avoid complication. METHODS: In our institute, 45 glioma patients underwent tumor resection using neuro-navigation system, brain mapping, and 5-ALA PD from 2013 to 2017. Evaluation of presence or absence of postoperative ischemic complication was measuring distance between removal cavity and ischemia lesion, referring diffusion-weighted MR image. And, evaluation of presence or absence of neurological deficits were referred to medical records. RESULTS: In all 45 cases, 30 cases (68%) appeared abnormal change in diffusion-weighted MR image, 7 cases (17%) of them had neurological deficits because of ischemic complication. All cases of within 5mm ischemic complication from removal cavity with MRI had not clinical neurological deficits. CONCLUSION: More than half our cases appeared ischemic complication. And it is considered necessary to undergo operation while thinking possibility of onset risk of ischemic complication. In particular, resection of brain tumor near eloquent area, we suggest one method of residual tumor 5mm from eloquent area because of prevent neurological function.

NIMG-79. EARLY TREATMENT RESPONSE ASSESSMENT USING O-(2-^{18}F-FLUOROETHYL)-L-TYROSINE (FET) PET COMPARED TO MRI IN MALIGNANT GLIOMAS TREATED WITH ADJUVANT TEMOZOLOMIDE CHEMOTHERAPY

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BACKGROUND: The goal of this prospective study was to compare the value of conventional MRI and O-(2-^{18}F-fluoroethyl)-L-tyrosine (FET) PET for response assessment in patients with malignant glioma treated with first-line adjuvant temozolomide chemotherapy (TMZ). METHODS: After biopsy/resection and completion of radiotherapy with concomitant temozolomide, 34 malignant glioma patients (glioblastoma, n=31; IDH-wildtype anaplastic astrocytoma, n=2; H3K27-mutated midline glioma, n=1) (age range, 20–66 years) were subsequently treated with adjuvant TMZ (5/28). FET-PET scans were performed at baseline and after 10–12 weeks. The first follow-up MRI after radiotherapy (9 ± 3 weeks) was compared with the early postoperative MRI. We obtained FET metabolic tumor volumes (MTV) and tumor/brain ratios (TBR). Threshold values of FET-PET parameters for treatment response were established by ROC analyses using the progression-free survival (PFS) ≤/>9 months as reference. MRI response assessment was based on RANO criteria. The predictive ability of FET-PET thresholds and MRI changes on early response assessment was evaluated subsequently concerning PFS using univariate survival estimates. RESULTS: Relative TBR changes were not predictive for a PFS>9 months ($P>0.05$), whereas the absolute MTV at follow-up significantly predicted a PFS>9 months ($P=0.016$; threshold, 14.5 ml). The relative MTV change enabled the most significant PFS prediction. Responders defined by relative MTV changes (threshold, ≤0%) had a significantly 2-fold longer PFS than non-responders (16 vs. 8 months, $P=0.003$). RANO criteria at the first follow-up MRI after radiotherapy were not predictive for a PFS>9 months ($P=0.260$). CONCLUSIONS: FET-PET appears to be useful for identifying responders to adjuvant TMZ early after treatment initiation.

NIMG-80. A RADIOMIC SIGNATURE AS A NON-INVASIVE PREDICTOR OF PROGRESSION-FREE SURVIVAL IN PATIENTS WITH LOWER-GRADE GLIOMAS

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OBJECTIVE: The aim of this study was to develop a radiomics signature for prediction of progression-free survival (PFS) in lower-grade gliomas and to investigate the genetic background behind the radiomics signature. METHODS: In this retrospective study, training (n = 216) and validation (n = 84) cohorts were collected from the Cancer Genome Atlas and Chinese Glioma Genome Atlas, respectively. For each patient, a total of 431 radiomics features were extracted from preoperative T2-weighted magnetic resonance images. A radiomics signature was generated in the training cohort, and its prognostic value was evaluated in both the training and validation cohorts. The genetic characteristics of the group with high-risk scores were identified by radiogenomic analysis, and a nomogram was established for prediction of PFS. RESULTS: There was a significant association between the radiomics signature (including 9 screened radiom-

ics features) and PFS, which was independent of other clinicopathologic factors in both the training ($P < 0.001$, multivariate Cox regression) and validation ($P = 0.025$, multivariate Cox regression) cohorts. Radiogenomic analysis revealed that the radiomics signature was associated with the immune response, programmed cell death, cell proliferation, and vasculature development. A nomogram established using the radiomics signature and clinicopathologic risk factors demonstrated high accuracy and good calibration for prediction of PFS in both the training (C-index, 0.69) and validation (C-index, 0.87) cohorts. CONCLUSIONS: PFS can be predicted non-invasively in patients with LGGs by a group of radiomics features that could reflect the biological processes of these tumors.

NEUROLOGICAL COMPLICATIONS OF CANCER AND CANCER THERAPY

NCMP-01. COMPARISON AND QUANTITATION OF HISTOPATHOLOGY ABNORMALITIES IN SURGICALLY RESECTED CEREBRAL RADIATION NECROSIS AS COMPARED WITH RECURRENT BRAIN TUMOR FOLLOWING RADIATION

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BACKGROUND The histologic features of radiation necrosis (RTN), a dysregulated repair process following brain radiation, have not been defined and distinguished from recurrent brain tumor (RBT) following brain radiation therapy. The aim of this study is to compare the type and severity of histologic characteristics of tissue specimens containing predominantly or totally RTN versus predominantly or totally RBT obtained at imaging progression after brain tumor radiation. METHODS Subjects were identified from brain tumor pathology reports of resected recurrent/progressing MRI enhancing lesions following brain radiation at UHCMC from 2004--2013. RTN was defined by < 20% active tumor, the remainder radiation treatment effects. RBT was defined by >80% active tumor. (Mixed cases were excluded). H & E slides were reviewed for 30 characteristics including vascular pathologies, necrosis, tumor features, tissue reaction, inflammatory infiltrate, blood products, and dystrophic calcification. Localization in grey/white matter and leptomeninges was noted. Each characteristic was graded in quartiles by one neuropathologist. The profile was compared with original tumor when available. RESULTS 66 patients were identified, 40 RBT (25 glioma, 15 metastasis) and 26 RTN (14 metastasis, 12 glioma). We identified significant differences in frequency and severity of zonal/geographic necrosis in RTN versus RBT in glioma (p=.002) and metastasis (p=.012) and nonsignificant difference in severity of vascular hyalinization in RTN versus glioma RBT (p=0.44). Demyelination was of borderline significance. Fibrinoid vascular necrosis, vessel wall thickening, and RT astrocytes were not significantly different in RTN versus RBT of either tumor type. In some cases, necrosis and vascular pathology were similar to pretreatment histology. CONCLUSION A multivariable assessment of histologic characteristics may assist neuropathologists in interpretation of surgically resected lesions following brain tumor radiation therapy, especially when pretreatment tumor is available for comparison. We will present imaging correlations with pathology findings. Grant support CTSC UL1TR000439

NCMP-02. MULTIPLE SCLEROSIS OUTCOMES AFTER CANCER IMMUNOTHERAPY

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INTRODUCTION: Multiple sclerosis as an adverse event of immune checkpoint inhibitors (ICI) is rare and the outcomes remain largely unknown with limited descriptions in the literature. METHODS: We analyzed the United States Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) database, our institutional records, and the literature for pembrolizumab, atezolizumab, nivolumab, ipilimumab, avelumab, and durvalumab two years prior their FDA approval to December 31, 2017, for all cases of newly diagnosed or MS relapse. RESULTS: We identified 14 cases; 11 cases from FAERS, 1 case was identified from our institution, and 2 cases from the literature. The median age was 52.5 years, with 42.8% males and 42.8% females. Indications for ICI included melanoma (6 patients), non-small cell lung carcinoma (2 cases), pleural mesothelioma (1 case), renal cell carcinoma (1 case), colorectal cancer (1 case), and was not reported in 2 cases. Two cases were identified from pembrolizumab use, 6 cases with nivolumab, 1 case with atezolizumab, and 5 cases after ipilimumab use. No cases were reported with the use of avelumab or durvalumab. History of multiple sclerosis was confirmed in 8 cases. Mean time to beginning of symptoms was 29 days (3 cycles). The median time to beginning of symptoms was longer in patients receiving ipilimumab (45 days, 4 cycles). Median time for symptom resolution in all the patients was 2 months. All cases required hospitalization, further treatment, and close follow up. Two patients died after starting therapy. CONCLUSIONS: MS may be associated with overall state deterioration, significant disability, and death. The benefits of ICI should be carefully assessed prior to beginning therapy in patients with a history of MS.

NCMP-03. RISK FACTORS FOR SURGICAL SITE INFECTIONS AFTER CRANIOTOMY FOR PRIMARY BRAIN TUMORS

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Surgical site infection (SSI) after craniotomy for primary CNS tumors can have detrimental consequences by delaying chemoradiation treatment. The authors performed a retrospective chart review of all patients who underwent craniotomies for resection of primary brain tumors at the Moffitt Cancer Center from 2004–2014. Multivariate logistic analysis was used to identify independent risk factors. A total of 864 patients underwent craniotomies for primary brain tumors, but 65 were excluded due to insufficient followup or incomplete records. We identified 30 patients with SSI (3.8%). The most common microorganisms isolated from SSI were methicillin resistant *Staphylococcus aureus* (40%), methicillin sensitive *Staphylococcus aureus* (17%), methicillin resistant *Streptococcus epidermidis* (7%), *Pseudomonas* (7%), *Enterobacteriaceae* (7%), and *E coli* (7%). During the latter part of this time period, we initiated a program of intraoperative topical vancomycin application. We observed a significant reduction in SSI among those receiving topical vancomycin compared to those without (0.8% vs 4.9%, p<0.001). The cohorts were similar in demographics and baseline comorbidities, KPS, tumor characteristics, and surgical factors. We identified length-of-stay, previous radiation and preoperative steroid dose as independent risk factors for SSI. Thus, our study identifies potential modifiable risk factors for the prevention of SSI in patient undergoing craniotomy for primary CNS tumors.

NCMP-05. RITUXIMAB AS INITIAL THERAPY FOR THE REVERSAL OF MYASTHENIA GRAVIS NEUROTOXICITY CAUSED BY IPILIMUMAB/NIVOLUMAB

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BACKGROUND: In patients with melanoma treated with immune checkpoint inhibitors (ICIs), neurological toxicities developed in 1% treated with Ipilimumab and 3% after Nivolumab. This rises to 14% when co-administered. Patients treated with ICIs are susceptible to Myasthenia Gravis like syndrome. There are several case reports that demonstrate the benefits of rituximab in both acetylcholine receptor (AChR) and muscle specific kinase (MuSK) antibody-positive MG patients. This study demonstrates the benefit of Rituximab in early goal therapy. OBSERVATION: A 70 year old female with scalp dermal spindle cell melanoma participating in a clinical trial with Nivolumab versus Ipilimumab/Nivolumab presented with diplopia. She suffered from shortness of breath and intermittent bilateral eye ptosis. Her ophthalmologic symptoms started following cycle one of therapy. Her ophthalmologist and allergist felt she had allergies. Her symptoms worsened with generalized muscle weakness and horizontal diplopia. One year later, her neurologist diagnosed her with Myasthenia Gravis. She did not receive treatment and had difficulty contacting her neurologist. She saw her pulmonologist for worsening dyspnea and her pulmonary function test showed a decreased negative inspiratory force (NIF) < 20 cm H2O. She declined admission and was treated with Mestinon 60 mg q4hr and prednisone 90 mg. Her NIF and FVC did not improve and she was given IVIG for 5 days. Her PFTs had only slightly improved. She received 1 dose of Rituximab at 375 mg/m2 and her FVC improved to >2.5 L. She was discharged on prednisone 40 mg daily and Mestinon 60 mg TID. Three days after Rituximab she was completely asymptomatic. DISCUSSION: Our case study shows a patient treated with immune checkpoint inhibitors developed Myasthenia gravis like syndrome and was successfully treated with Rituximab. CONCLUSION: Our study shows the importance of Rituximab as initial therapy for the reversal of MG like syndrome caused by Ipilimumab/Nivolumab.

NCMP-07. CLINICAL NEUROLOGICAL FEATURES AND ELECTROGRAPHIC PATTERNS OF PATIENTS WITH RELAPSED OR REFRACTORY LARGE B-CELL LYMPHOMA TREATED WITH AXICABTAGENE CILOLEUCEL AT MEMORIAL SLOAN KETTERING CANCER CENTER (MSKCC)

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CD19-specific chimeric antigen receptor (CAR) T cell therapy is a promising treatment for hematological malignancies however is associated with neurotoxicity. Axicabtagene ciloleucel is approved to treat adult patients with relapsed or refractory large B-cell lymphoma. Here we describe the neurological features and encephalogram (EEG) patterns of patients with refractory lymphoma treated with axicabtagene ciloleucel at Memorial Sloan Kettering Cancer Center (MSKCC). We retrospectively analyzed the neurological features and EEG patterns of the first six patients with refractory lymphoma treated at MSKCC with axicabtagine ciloleucel between February and April 2018. All six patients developed neurotoxicity, with both diffuse encephalopathy and focal neurological features.None of the patients had pre-existing neurological conditions. All patients received prophylatic levetiracetam and had a long-term video EEG performed. Two patients developed neurological findings concerning for focal status epilepticus. One patient had rhythmic right arm movements followed by weakness and the EEG showed left lateralized rhythmic delta activity (LRDA) with sharp waves and attenuation possibly reflecting a post-ictal state. The second patient had right facial and bilateral eye twitching concerning for focal motor status epilepticus. The EEG showed diffuse dysfunction - rhythmic delta activity and triphasic waves. Both patients had clinical improvement with addition of fosphenytoin. The remaining four patients had EEGs showing diffuse cerebral dysfunction. Neuroimaging in all patients was unrevealing for acute or significant structural pathology. Most patients had resolving neurologic symptoms; one patient had prolonged paraparesis. CONCLUSIONS: Axicabtagene ciloleucel can lead to transient diffuse and focal neurological findings of varying severity including focal status epilepticus. EEG is useful in characterizing cerebral dysfunction however is limited in detecting epileptic events. In patients who develop clinical status epilepticus escalation of AEDs may be warranted despite EEG not fulfilling status epilepticus criteria. Further study is needed to determine whether AED escalation can optimize patient outcomes.

NCMP-08. MANAGEMENT OF RADIONECROSIS AFTER STEREOTACTIC RADIATION FOR BRAIN METASTASES IN THE SETTING OF IMMUNE CHECKPOINT INHIBITORS

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BACKGROUND: Immune checkpoint inhibitors (ICI) are an increasingly common therapy for metastatic solid tumors. Despite their efficacy, many patients develop brain metastasis requiring stereotactic radiosurgery (SRS) or fractionated stereotactic radiation therapy (SRT). Radiation necrosis (RN) is a known risk. Recent observations suggest that patients receiving SRT/ICI may experience synergism albeit with potential increased risk of symptomatic RN. The best treatment in this setting remains unknown. VEGF inhibitors have demonstrable efficacy in treatment of RN and may be beneficial in treating neurotoxicity associated with combination SRT/ICI. METHODS: Data were analyzed retrospectively from 169 consecutive patients at a single institution undergoing SRS/SRT for brain metastasis from 2015–2017. 10 patients were eligible for evaluation having received SRT and ICI within a 6 month timeframe and with a minimum 3 months survival for assessment of RN. 7 patients were treated for lung adenocarcinoma, 2 for high-grade neuroendocrine tumor, and 1 with triple-negative breast cancer. RESULTS: 10 patients were treated with SRS (90%), SRT (60%), both (50%), or SRS followed by whole-brain radiation (10%) to a total of 65 brain metastases. ICI (nivolumab, pembrolizumab, atezolizumab, ipilimumab) was administered before, during, or after SRS/SRT. 6 patients (60%) developed symptomatic grade ≥ 1 neurological adverse events. Of these 6, 4 (40%) developed grade 3 RN requiring bevacizumab. Symptoms resolved rapidly in 3 of 4 patients (75%), the 4th patient died of complications of disease shortly after bevacizumab administration. Mean 12 month survival from initial immunotherapy treatment was 89% and mean 18 month survival from initial SRT was 78%. CONCLUSION: Combination treatment with SRT/ICI for brain metastases appears to be associated with high rates of RN compared to historical standards. Given the prolonged survival seen, appropriate management of this complication is paramount. Short course bevacizumab appears highly effective in treating symptomatic RN associated with ICI. Further evaluation with a larger sample size is warranted.

NCMP-09. ISOCITRATE DEHYDROGENASE MUTATIONS AND INCREASED TISSUE 2-HYDROXYGLUTARATE CONCENTRATION MIGHT BE RELATED WITH SEIZURE ONSET IN PATIENTS WITH GLIOMAS

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Mutations in isocitrate dehydrogenase 1 or 2 genes (IDH1/2) frequently occur in lower-grade gliomas and secondary glioblastomas. Mutant IDH1/2 proteins gain a new ability to produce the oncometabolite 2-hydroxyglutarate (2HG). Recently, IDH1/2 mutations were suggested to be related with seizure onset through the structural similarity of 2HG to the excitatory neurotransmitter glutamate. Therefore, we sought to investigate the relationship between seizure onset and IDH1/2 status, tissue 2HG concentration and distribution. We assessed 149 patients with WHO grade II-IV glioma, whose IDH1/2 status were identified and measured serum and tissue 2HG concentrations in 123 and 104 patients by using liquid chromatography-tandem mass spectrometry method. The matrix assisted laser desorption and ionization mass spectrometry imaging (MALDI-MSI) was use to analyze tissue 2HG distribution for 12 tissue samples. Seizure onset was observed in 34 among 56 (60.7%) patients with IDH mutant tumor, whereas in 18 among 93 (19.4%) patients with IDH wild-type tumor ($p<0.0001$). The median tissue 2HG concentrations in patients with seizure onset were significantly higher than in those without seizure onset (2860mg/mg vs 110mg/mg, $p<0.0001$). Receiver operating characteristic analysis suggested the cutoff value of the tissue 2HG concentration was of 1190mg/mg. Multivariate analysis, including tissue 2HG concentration, serum 2HG concentration, IDH1/2 mutation, tumor histology, WHO grade, tumor location, and patient age, suggested that tissue 2HG concentration and serum 2HG concentration were significantly correlated with seizure onset. The MALDI-MSI showed heterogeneous 2HG distribution in IDH mutant sections. Our results suggest that increased tissue 2HG concentration and heterogeneous distribution might be related to seizure onset in patients with gliomas.

NCMP-10. AUTOIMMUNE ENCEPHALITIS IN POST-REMISSION PINEAL GERMINOMA: A CASE REPORT.

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INTRODUCTION: Autoimmune encephalitis is a well-characterized complication of gonadal and extragonadal germ cell tumors (GCT), with a known culprit: anti-N-methyl-D-aspartate receptor antibodies. However, reports of autoimmune encephalitis in patients with central nervous system (CNS) GCT are rare. Here, we describe a case of progressive, severe encephalopathy of unknown etiology in a patient with history of pineal germinoma that responded to immunosuppressive therapy. CASE DESCRIPTION: An 18-year old female with history of pineal germinoma presented with a two month history of worsening confusion, short-term memory loss and nocturnal enuresis three months after achieving complete response following chemoradiotherapy. She denied fevers, headaches, ingestions or trauma. While admitted, her level of consciousness acutely deteriorated, necessitating intubation and mechanical ventilation. During this time, her exam was significant for episodic decerebrate posturing with associated dysautonomia. An extensive workup, including infectious, neoplastic and paraneoplastic testing, was nondiagnostic. An autoimmune encephalitis panel sent to a reference laboratory was negative. Electroencephalography was consistent with severe encephalopathy without evidence of seizure activity. She was treated empirically with plasma exchange, intravenous immune globulin and rituximab, demonstrating a striking return to baseline level of consciousness and extinction of dysautonomia. Nine months after rituximab treatment, she developed headaches, dizziness, diplopia and memory loss. She was treated again with rituximab and had full recovery. DISCUSSION: Autoimmune encephalitis is a known complication in cases of gonadal and extragonadal GCT, characterized by confusion, memory deficits and dysautonomia. However, little literature exists regarding this phenomenon in the genetically indistinguishable CNS GCT. Here, we describe the case of a young woman with history of pineal germinoma who presented with severe encephalopathy. Though no specific agent has yet been identified, the resolution of her symptoms after empiric treatment for autoimmune encephalitis suggests that clinicians should be cognizant of this potential complication in patients with CNS GCT.

NCMP-11. NEURODEGENERATION AND CHEMOBRAIN: PREDICTING THE PROBABILITY OF ALZHEIMERS DISEASE IN BREAST CANCER SURVIVORS

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Chemotherapy treatment for cancer has been associated with significant alterations in brain structure and function that result in cognitive impairments. Many of the candidate mechanisms for chemotherapy-related brain injury are closely associated with aging. Therefore, it has been suggested that chemotherapy may alter or even accelerate the brain aging trajectory. Accordingly, certain epidemiological studies have suggested that chemotherapy treated patients are at higher risk for pathologic neurodegenerative processes such as Alzheimers disease (AD). Other studies have failed to replicate these findings so empirical evidence for increased AD risk following chemotherapy remains limited. In this retrospective study, we obtained brain MRI data from 47 females age 69 +/- 7 years who were later diagnosed with AD and 47 matched female controls who did not develop AD. Using random forest classification, we created a model of brain network organization, APOE genotype and age that could successfully discriminate between AD converters and controls with 86% accuracy ($p < .0001$, ROC = .96). We then applied this model to data obtained from 78 breast cancer survivors to calculate predicted probability of AD for each individual. Chemotherapy-treated survivors demonstrated significantly higher probability of AD compared to chemotherapy naïve survivors ($p = .007$) even after stratifying for APOE e4 genotype ($p = .014$). Cognitive dysfunction was significantly associated with higher AD probability, older age and chemotherapy treatment ($r = .74$, $p = .04$). These findings suggest that chemotherapy-treated survivors who have a particular profile of brain network organization may be at higher risk for AD, regardless of other risk factors including age and genotype.

NCMP-13. BDNF ENHANCEMENT VIA AMPAKINES AS A POTENTIAL TREATMENT FOR CHEMOTHERAPY-RELATED COGNITIVE IMPAIRMENT

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OBJECTIVES: Chemotherapy-related cognitive impairment (CRCI) is widely reported among cancer survivors. We focus on the neurocognitive impairments provoked by cisplatin, which is used for treatment of various malignancies including ovarian, testicular, head and neck cancers, and pediatric brain tumors. More than 30% of advanced ovarian cancer patients develop CRCI during and after cisplatin-based chemotherapy. We examined the role of brain-derived neurotrophic factor (BDNF) downregulation in cisplatin-induced CRCI, and whether pharmacological BDNF augmentation via ampakines can prevent cisplatin-induced neuronal damage. BDNF is broadly expressed in the hippocampus where it regulates dendritic spine integrity and neurogenesis, among other functions. METHODS: We examined the effects of cisplatin on neurogenesis, neuronal morphology, BDNF levels, and cognition in rats. We also assessed the effects of cisplatin on dendritic spine density, BDNF mRNA levels, apoptosis, and the ability of ampakine CX1739 to mitigate cisplatin-induced neuronal damage in-vitro. RESULTS: Cisplatin reduced dendritic branching and spine density, and neurogenesis in the rat hippocampus. Chronic cisplatin treatment decreased hippocampal and serum BDNF levels, and impaired cognitive function in rats. In-vitro, ampakine administration upregulated BDNF levels, and mitigated cisplatin-induced dendritic damage in cultured hippocampal neurons. Concomitant administration of CX1739 with cisplatin did not affect cisplatins cytotoxic activity in two ovarian cancer cell lines. DISCUSSION: The cognitive deficits caused by cisplatin in rats result from the loss of excitatory synapses and dendritic spines that anchor them, neuronal apoptosis, as well as decrease in neurogenesis. Changes in BDNF levels are associated with cisplatin-induced CRCI. Importantly, CX1739 mitigated cisplatin-induced neurotoxicity and BDNF depletion in vitro. Next, we will examine whether CX1739 prevents cisplatin-induced cognitive deficits in rats. If so, we will plan a Phase I study to examine if ampakine administration to ovarian cancer patients receiving cisplatin is safe, and if it ameliorates the cognitive deficits described in this patient population.

NCMP-14. TREATMENT RESPONSE OF LEPTOMENINGEAL CARCINOMATOSIS IN CLL: A CASE REPORT AND REVIEW OF THE LITERATURE

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INTRODUCTION: Leptomeningeal carcinomatosis is a rarely-reported complication of chronic lymphocytic leukemia (CLL), and its significance is debated. We report a case of leptomeningeal CLL presenting with lower extremity weakness with excellent clinical response to ibrutinib and later to intrathecal rituximab. METHODS: A comprehensive review was performed encompassing 103 published cases of leptomeningeal disease in CLL since 1976. Emphasis was placed on treatment strategy and response, and the clinical significance of CSF clearance. RESULTS: When presenting symptoms were grouped by effect of leptomeningeal carcinomatosis on three anatomic sites (1) cerebral or cerebellar parenchyma, 2) nerve roots, and 3) intracranial pressure due to impaired CSF absorption by arachnoid granulations, 99% of patients had symptoms from at least one category and 40% had symptoms from multiple categories. OS was >14.3 months after diagnosis of leptomeningeal carcinomatosis, and this was unaffected by Rai stage at presentation. All patients treated with ibrutinib (N=7) achieved complete remission (CR), whereas other chemotherapies had failure rates ranging from 7% (intrathecal methotrexate, N=55) to 50% (vincristine, N=10). When best overall response to treatment was scored, 76 patients achieved CR, 7 patients achieved PR, 10 patients were not treated, and 10 patients had no response. CSF clearance was observed in 64 cases with median OS 12 months, failure of clearance was reported in 21 cases with median OS 2 months, and 14 cases did not report CSF response. CONCLUSIONS: We confirm that symptoms of leptomeningeal carcinomatosis in CLL are reversible and leptomeningeal disease is associated with a more favorable OS in CLL compared to other cancers. Presenting symptoms follow a clear and predictable pattern. Clearance of CSF with therapy is not required for symptomatic improvement but is associated with increased OS. Finally, ibrutinib and rituximab were the chemotherapies most frequently associated with complete response.

NCMP-15. POSTOPERATIVE CEREBELLAR MUTISM SYNDROME FOLLOWING TREATMENT OF MEDULLOBLASTOMA: NEUROLOGICAL CHARACTERISTICS AND SEVERITY BY MOLECULAR SUBTYPE

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OBJECT: To examine the neurological characteristics and severity of cerebellar mutism syndrome (CMS) in pediatric medulloblastoma (MB) post-operatively and at one-year post-surgery by MB molecular subtype. METHODS: We obtained Institutional Review Board (IRB) approval and retrospectively reviewed 146 patients 18 years old with MB treated on SJMB03 protocol at our institution from 2000–2010 for clinical, surgical, radiographical information, molecular subtype, and history of CMS. CMS neurological characteristics and severity were recorded from pre-operative and at 1 year follow-up post-operative times and scored using National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) version 4.0, which scores 0 (absent) to 5 (death) for symptoms. CMS characteristics and severity immediately post-resection were scored using the Robertson scoring system, which scores mild (<1 week), moderate (1–4 weeks), and severe (>4 weeks). RESULTS: Forty-two (28.7%) of 146 patients had CMS. Twenty-four (57.1%) of CMS patients were Group 4 subtype ($P<0.03$). CMS significantly correlated with midline intraventricular tumor location in 39 (92.9%) of CMS patients ($P<0.04$). Damage to both the dentate nuclei and superior cerebellar peduncles significantly correlated to CMS ($P<0.0001$ and $P<0.0001$, respectively). Bilateral damage to the efferent cerebellar pathway significantly correlated in 26 (61.9%) CMS patients ($P<0.0001$). Initial post-operative severity of eye movement disorders significantly correlated with severity at one year follow up in all patients ($P<0.04$) and trended in group 4 subtype tumors severity at one year follow ($P<0.07$). CONCLUSIONS: Group 4 subtype MB tumors and midline intraventricular tumor location are both significant risk factors for CMS. Post-operative MRI findings of bilateral efferent cerebellar pathway damage, dentate nuclei damage, and superior cerebellar peduncle damage are also risk factors for CMS. In all patients as well as group 4 subtype, initial post-operative severity of neurological findings do not correlate with severity at one year follow up except for eye movement disorders.

NCMP-16. RADIATION RECALL MYELITIS FOLLOWING PACLITAXEL CHEMOTHERAPY IN METASTATIC BREAST CANCER WITH PRIOR SPINAL STEREOTACTIC BODY RADIOTHERAPY: THE FIRST REPORTED CASE

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INTRODUCTION: Stereotactic body radiotherapy (SBRT) of the spine has become an increasingly utilized modality in the United States, most commonly for metastatic disease (McClelland et al., 2017). Spinal SBRT in patients with spinal instrumentation has been sparsely examined. We report a patient who developed myelitis following spinal SBRT to a region with existing hardware. METHODS: A 55-year-old woman with Stage IV breast

cancer developed a T4 vertebral body metastasis and underwent tumor debulking with posteriorly instrumented T3-T5 fusion. Postoperatively she proceeded with SBRT to the T3-T5 vertebral bodies, receiving 30 Gy in 6 Gy/fraction. Six months later, she underwent palliative RT for a new right clavicle metastasis (20 Gy in 4 Gy/fraction). She subsequently required paclitaxel chemotherapy for new liver metastases. RESULTS: Seven months following spine SBRT, shortly after having started chemotherapy she developed intractable back pain and right lower extremity numbness which improved upon receiving steroids for weekly chemotherapy; the numbness subsequently spread to her left leg. Thoracic spine MRI revealed a 1.7 cm ovoid focus of T4-T5 spinal cord enhancement with extensive surrounding cord edema extending superiorly to C6-C7, consistent with radiation myelitis. Hyperbaric oxygen moderately improved her symptoms; fortunately, she never developed motor symptomatology or bowel/bladder dysfunction. Thorough re-evaluation of the original thoracic spine SBRT plan revealed no deviations from the standard of care, nor did re-planning with alternate treatment planning software demonstrate any significant difference in maximum cord dosage than the original plan. CONCLUSIONS: The timing of symptomatology related to chemotherapy administration is consistent with radiation recall myelitis, which has yet to be reported following SBRT. Given the potentially disastrous consequences of myelitis, patients with metastatic disease previously treated with spine SBRT may be susceptible to developing radiation recall myelitis if treated with paclitaxel chemotherapy.

NCMP-17. EVOLUTION OF CEREBRAL MICROBLEEDS AFTER PROTON IRRADIATION IN LOW-GRADE GLIOMA PATIENTS

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BACKGROUND: Cranial irradiation is associated with neurovascular injury, such as cerebral microbleeds (CMBs). We have previously shown that CMBs progressively increase in number with each year after completion of photon-based radiation therapy (RT). We hypothesize that proton RT may be associated with reduced microvascular injury given its limitation of radiation exposure to the treatment field, To explore the temporal and spatial pattern of CMBs after proton RT, we followed a cohort of glioma patients using longitudinal imaging over a 10-year period. METHODS: 20 patients with low-grade glioma were treated with proton RT (54 Gy, 1.8Gy/fx). The occurrence of CMBs was evaluated longitudinally using T2* gradient echo imaging and susceptibility-weighted imaging at baseline and up to 10 years post RT. The temporal and spatial distribution patterns of CMBs were characterized. RESULTS: The mean age at time of RT was 38.2 years (SD 8.8 yrs) in this cohort of 20 patients (13 male, 7 female). Patients were followed for a median time of 7.5 yrs (range 1.5–10). CMBs were detected with an incidence of 40% at 1 yr, 71% at 2 yrs, 94% at 5 yrs and 100% at 10yrs. The average cumulative number of CMBs per patient was 0.67 at 1 year, 2.5 at 2 yrs, 8.9 at 5 yrs and 12.5 at 10 yrs. CMBs occurred in lobar (83.4%), deep territory (11.5%) and infratentorial distribution (5%). One patient developed disseminated cortical superficial siderosis 3 years after RT and 3 patients developed intracerebral hemorrhage between 4 and 8 yrs after RT. CONCLUSIONS: In this cohort of low-grade glioma patients and similar to photon irradiated patients the prevalence of CMBs increases with each year following proton RT and mostly follows a lobar distribution pattern. CMBs are usually only detected within the main radiation field but can be associated with intracerebral hemorrhage.

NCMP-18. PLATELET COUNT IS ASSOCIATED WITH OUTCOME IN CANCER PATIENTS STROKE

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INTRODUCTION: Cerebrovascular disease (CVD) and cancer are among the most common causes of mortality worldwide, preceded only by ischemic heart disease (IHD). Thrombocytopenia was shown to be associated with poor outcomes in IHD and CVD in the general population. This study aimed to assess the relationship of thrombocytopenia with poor outcomes in cancer patients with CVD. MATERIALS AND METHODS: Data on patients with concomitant CVD and cancer who were initially treated at a cancer referral center between January 2010 and December 2017 were included. Thrombocytopenia was defined as a platelet count <150,000/mm3 during the first 24 h of CVD symptom onset. The review of clinical records was approved by the IRB. RESULTS: Among 268 cancer patients with CVD included in the study, 210 met the inclusion criteria. Median overall survival of the entire cohort was 7.2 months, which was significantly shorter in males (p 0.029) and patients with hematologic tumors (p = 0.009), hemorrhagic CVD (p < 0.001), altered mental status (p < 0.001), and thrombocytopenia (p < 0.001). Multiple regression logistic analysis revealed that thrombocytopenia (risk ratio [RR] 1.6, 95% confidence interval [CI] 1.12.4) and altered mental status (RR 2.7, 95%CI 1.94.0) remained statistically significant risk factors for mortality. CONCLUSION: In cancer patients with CVD, thrombocytopenia at the time of CVD diagnosis and altered mental status during initial clinical evaluation were associated with higher mortality, which should be confirmed in future studies.

NCMP-19. PERIOPERATIVE SEIZURE OF BRAIN TUMOR SURGERY AND ITS PREVENTION.: A SINGLE INSTITUTE EXPERIENCE

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Standard care for prevention of perioperative seizure in brain tumor surgery is not established and prophylactic use of antiepileptic drug in seizure-naïve patients is controversial. To examine the efficacy and problem of perioperative antiepileptic therapy, we retrospectively studied brain tumor patients who received surgical treatment in our institution between July 2016 and May 2018. Among 128 patients who underwent tumor resection or biopsy, sixty-five (50.1%) were received antiepileptic drug therapy. Twenty-six patients had preoperative seizure (Group A). Histopathological diagnoses of Group A were metastatic brain tumor (11 patients: 42.3%), meningioma (7 patients: 26.9%), gliomas (5 patients: 19.2), and malignant lymphoma (3 patients: 11.5%). Eighteen patients (69.2%) of them were treated with levetiracetam (LEV) monotherapy, followed by valproic acid (VPA). Thirty-eight patients (29.7%) received prophylactic use (Group B). Histopathological diagnoses of Group B were 9 metastases (23.7%), 18 meningiomas (47.4%), 9 gliomas (23.7%), and 2 malignant lymphomas (0.5%). Thirty-four patients (87.2%) were initially treated with LEV, followed by phenytoin, and VPA was not used. We treated Group A patients with same manner as before surgery, and convulsion occurred 6 patients (23.1%) within a week after surgery. Three patents were added other drugs and all were successfully treated after seizure. On the other hand, no seizure was observed in Group B. Mild adverse effect (dizziness) occurred in two patients in Group B. One was reduced drug amount and the other was changed drug. Recently, novel antiepileptic drugs have been introduced and they can be used in Japan. Further study will be needed to lower the seizure rate in perioperative period of tumor burden patients by those drugs. Complete seizure prevention and low complication would allow prophylactic treatment on basis of our institutional strategy.

NCMP-20. TUMOR TYPES, RESECTION AND INCIDENCE OF NEUROPATHY: CURRENT LITERATURE REVIEW

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PURPOSE: By identifying the individual sub-categorical phenotypes most associated with neuropathy and collectively using to specify a single tumor most associated with neuropathy, the hopes of this literature review are to help clinicians begin treatment for neuropathy before symptoms become severe and perhaps prevent it to the best of their ability. METHODS: All clinical studies and literature reviews analyzed in this review were obtained through PubMed Central from 1990 through 2017. Characteristics included shape, size, location, approximate estimation of growth period, presence and occurrence of metastasis, and original location of tumor. After a thorough consideration of these characteristics, the specific phenotypes of the tumor for each of these categories most likely to result in any form of neuropathy was determined. Furthermore, the individual subcategory phenotypes were then aggregated to characteristically specify a single tumor most associated with severe neuropathy. RESULTS: The most commonly affected regions of peripheral nerves were analyzed to give better idea of which regions of tumor incidence would be most affiliated with an eventual onset of neuropathy. So certain degree of preventative measures and follow-up with the patient frequently due to the knowledge that neuropathy is very likely to occur. Certain chemotherapies were found to be associated with the most cases of neuropathy. Lung and Breast was found to lead to the most cases of neuropathy. These two cancers were also found to be most associated with brain metastasis which further sheds light on why neuropathy and paraneoplastic disease is occurring in patients with these forms of cancer. CONCLUSIONS: Through a detailed analysis of cancers most associated with neuropathy and paraneoplastic disease, a certain guidelines and risk factors were generated to help prepare for neuropathy following diagnosis of cancers. The warning guideline includes form of chemotherapy, the presence of lung or breast cancer, signs of brain metastasis.

NCMP-21. REAL-WORLD SURVEILLANCE DATA FOR TUMOR TREATING FIELDS AFFIRM THE TOLERABILITY OF TUMOR TREATING FIELDS FOR THE TREATMENT OF GLIOBLASTOMA IN THE UNITED STATES

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INTRODUCTION: Tumor treating fields (TTFields) are a non-invasive, anti-mitotic therapy for the treatment of recurrent and newly diagnosed glioblastoma (GBM) and were approved for each indication in the United States (US) in 2011 and 2015, respectively, based on efficacy and safety data from the EF-11 and EF-14 clinical trials). In both trials, the most common adverse effect (AE) associated with TTFields therapy were mild to moderate skin toxicity underneath the transducer arrays of the Optune device. US post-marketing surveillance data collected for recurrent and newly diagnosed GBM patients treated with TTFields were analyzed to assess the real-world safety profile of TTFields for GBM. METHODS: A review of the clinical information for recurrent and newly diagnosed GBM treated with TTFields in the United States identified 6,494 patients. The safety data obtained from post-market surveillance in all 6,494 patients were analyzed to identify AEs based on the MedDRA body system (system organ class) preferred terms. RESULTS: Forty five percent (2,941/6,494) of GBM patients experienced at least 1 AE while receiving TTFields treatment. Patients reporting general AEs include: 9% electric sensations, 4% fatigue, 9% heat sensation and 5% pain reported pain in any location. Nervous system AEs included headache (6%) and seizure (8%). The most common AE were skin reactions that were reported by 1,807 patients (28%). CONCLUSIONS: Analysis of real-world safety data for TTFields treatment in the US did not reveal any new safety signals for TTFields in the treatment of recurrent or newly diagnosed GBM. The most common AE related to TTFields were skin irritation under the transducer arrays. Skin reactions can be managed with topical treatments and slight adjustment of the transducer arrays to minimize skin irritation. Analyses of post-marketing US safety data corroborate the tolerability of TTFields for GBM patients observed in the Phase 3 clinical trials.

NCMP-22. TREATMENT-RELATED ADVERSE EFFECTS IN PATIENTS WITH MALIGNANT GLIOMA: ESTABLISHMENT OF KEY FEATURES FOR PSEUDOPROGRESSION AND TREATMENT-INDUCED NECROSIS.

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OBJECTIVE: Pseudoprogression (PP) and treatment-induced necrosis (TN) are insufficiently characterized, clinically challenging conditions. Since both entities radiographically mimic recurrent disease, patients frequently require surgical interventions to guide management. We aimed to characterize the clinical and radiographic features of PP and TN that may facilitate non-invasive differentiation from recurrent disease. METHODS: Patients with malignant glioma and a diagnosis of either PP (appearance <6 months post-radiotherapy [RT] completion) or TN (appearance >6 months post-RT) were retrospectively identified and compared using clinical-radiographic and histopathological data. Each imaging event/lesion diagnosed as PP or TN was evaluated as a region of interest (ROI) by T1+C weighted MRI and correlated to the respective RT dose distribution. RESULTS: Cumulatively, PP (n=27) and TN (n=37) groups comprised 137 individual radiographic ROIs (n=62 biopsy-proven, n=75 radiographic diagnosis). Most patients received concurrent and sequential chemotherapy. Gender and KPS did not differ significantly between groups. Patients with PP had mostly glioblastomas (81 vs 40%; p<0.002), fewer IDH1 mutations (p<0.006), a greater incidence of recurrence (p=0.03) and a shorter median overall survival (3.25 years vs. not reached (62% survival estimate at 24.5 years); p<0.0001). PP lesions occurred earlier (median onset post-RT: 1 vs. 11 months; p<0.00001), mostly during anti-neoplastic treatment (85 vs 32%; p<0.0005), and necessitated more steroid-based interventions (p<0.04). TN lesions often initially appeared periventricularly (n=22/37; 60%), were more numerous (median: 2 vs. 1 ROIs; p=0.01), and contained fewer malignant elements upon biopsy (p=0.008). While distance from the tumor resection cavity (RC) varied considerably for TN lesions (median: 21.5mm; range: 078mm), PP predominantly developed at the RC as a non-nodular, ring-like enhancing structure (p<0.0001). CONCLUSIONS: PP and TN appear to occur in clinically distinct patient populations and differ significantly in spatio-temporal radiographic pattern and histopathology. Increased familiarity with their unique features will improve patient management and may avoid unnecessary surgical procedures.

NCMP-23. FACIAL PALSY INDUCED BY CANCER IMMUNOTHERAPY: A SINGLE CENTER RETROSPECTIVE STUDY

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Monoclonal antibodies to Cytotoxic T-lymphocyteassociated antigen 4 (CTLA-4) [ipilimumab] and programmed cell death (PD1) receptor or ligand (PDL1) [pembrolizumab, nivolumab, atezolizumab] are increasingly used for the treatment of metastatic cancer. The augmented immune response enabled by these agents leads to the emergence of a class of side effects called immune-related adverse effects (irAEs) manifesting as autoimmune-like diseases. Neuromuscular complications such as polyneuritis, Guillain Barré syndrome (GBS)and myasthenia gravis are the most common; facial paralysis is only rarely reported. In this retrospective study, we reviewed the records of 364 patients who underwent immunotherapy in our center, for facial paralysis and Bells palsy, and identified five patients: 4 males and 1 female, ages 39 to 68 (average 55 years old) at the time of occurrence of facial paresis. Four patients had metastatic melanoma, all were treated with ipilimumab, in combination with nivolumab or pembrolizumab in 3 and 1 patients, respectively; the remaining patient had metastatic bladder and was treated with atezolizumab. Facial paralysis occurred within 1–23 weeks after starting immunotherapy and was unilateral in four patients. One patient had a multifocal neuropathy affecting limb and multiple cranial (including right facial) nerves; unilateral facial paresis emerged during the course of a GBS like condition in another patient. Lymphocytic pleocytosis was seen in CSF of three patients who had a lumbar puncture, MRI showed enhancement of the intracranial portion of the affected facial nerve in 4 patients. All but one patient, who had concomitant herpes labialis, were treated with steroids, with complete or significant improvement in 4; in the 5th patient facial weakness was still present after 3 months, before being lost to follow up. CONCLUSION: Facial paralysis is a less frequent neurological irAE, 1.4% in our cohort, and usually has a good prognosis.

NCMP-24. BRAIN-DERIVED CIRCULATING DNA AS A BIOMARKER FOR RADIOTHERAPY-INDUCED BRAIN DAMAGE

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INTRODUCTION: Radiotherapy is a common treatment for brain metastases. However, it is commonly associated with central nervous system (CNS) toxicity. There are no biomarkers for early detection of radiotoxicity. Here we explore the utility of cell-free circulating DNA (cfDNA) for detection of brain cells death in the context of brain metastases. Using comparative methylome analysis we have previously identified 12 genomic loci showing brain-specific DNA methylation patterns, including markers for neurons, oligodendrocytes and astrocytes. These brain-specific methylation markers were identified in plasma of patients suffering from multiple sclerosis, as well as traumatic and ischemic brain damage. We hypothesize that brain-derived cell-free DNA (bncfDNA) can be identified in patients suffering from brain metastases receiving radiotherapy, and can potentially be used as a biomarker to help guide treatment. MATERIALS AND METHODS: We recruit oncological patients treated by brain radiotherapy for brain metastases. We serially assess each patient before, during and after treatment by neurological examination and MRI studies. In each study visit a blood sample is collected for bncfDNA measurement. RESULTS: Preliminary results on samples from 22 patients show elevation of bncfDNA in patients suffering from brain metastases (average 8501 copies/ml; range 0-112336 copies/ml) compared with an extremely low background in healthy individuals (average 6 copies/ml; range 0–33 copies/ml); p < 0.0001). We observed elevation of cfDNA derived from neurons, oligodendrocytes and astrocytes. Next, we studied bncDNA levels following brain radiotherapy. Preliminary results from a patient suffering from an acute central facial paralysis during radiotherapy show clinical correlation of bncfDNA levels with neurological impairment related to acute radiotoxicity. Other patients are still followed-up with bncfDNA measurement during and after radiotherapy. CONCLUSION: BncfDNA reflects brain cells death incurred by metastases, as well as damage associated with radiotherapy, and may serve as circulating biomarker for neurotoxicity in patients suffering from brain metastases.

NCMP-25. SEIZURE INCIDENCE AND CONTRIBUTING FACTORS IN PATIENTS WITH LEPTOMENINGEAL DISEASE

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INTRODUCTION: Seizures are a well-known complication of CNS malignancy, however there is little in the literature regarding leptomeningeal disease (LMD) and seizures. The incidence of seizures is unknown in this specific cohort as is the use or potential benefit of preventative anti-seizure medications (AEDs). Additionally, factors that might predispose LMD patients to seize or affect their survival are largely unexplored. METHODS: Retrospective review of 79 patients with a diagnosis of LMD treated at a single institution from August 2012 to August 2017. Associations between categorical variables were tested using Fisher's Exact tests. Differences in survival between groups were plotted with Kaplan Meier curves and tested using log-rank tests. All analyses were performed using SAS software. RESULTS: Seizure incidence in those with and without brain metastases was 22%. Of those who seized, 65% were admitted for this at least once while only one patient required intubation. Primary malignancy, type or route of chemotherapy administration, form of radiation therapy (craniospinal, focal, or whole brain), and number of brain metastases did not influence seizure development. Only 8% of patients who never had seizures were on a prophylactic AED. In patients who had brain metastasis, there was no significant difference in incidence of seizure before vs after LMD diagnosis suggesting that LMD does not significantly increase the risk of seizure compared to brain metastasis alone. There was additionally no significant difference in survival time between patients who did or did not seize. Median survival time of patients after LMD diagnosis was 4 months. CONCLUSION: The incidence of seizure in LMD patients is 22%. There were no statistically significant predisposing factors to seizure development. Additionally, the development of seizures does not affect survival in patients with LMD.

NCMP-26. STROKE-LIKE MIGRAINE ATTACKS AFTER RADIATION THERAPY SYNDROME IN CHILDREN WITH CANCER

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BACKGROUND: Stroke-like migraine attacks after radiation therapy (SMART) syndrome is an infrequently described symptom complex of transient neurologic deficits, headache and abnormal cortical contrast enhancement on brain MRI. Pathophysiology is unclear, but exposure to cranial radiation (RT) is a sine qua non. METHODS: We completed a single institution retrospective case series composed of five children with a diagnosis of cancer, history of cranial RT, and episodes of transient neurologic deficits. RESULTS: Five children (2 males, 3 females) fulfilled diagnostic criteria. Tumors were in the posterior fossa (3 medulloblastoma, 1 atypical teratoid rhabdoid tumor) and temporal lobe (1 pleomorphic xanthoastrocytoma). Median age at diagnosis was 9.4 years (range 5.1–14.7). All patients had complete resection, followed by adjuvant 54 Gy focal RT (N=1) or 36 Gy CSI followed by a cone-down to 54 Gy (N=4), and chemotherapy. Median body mass index was 17.1 (range 14 to 30). Median time from the end of RT to first transient neurologic deficit was one year (range 0.7–12.1). Presenting symptoms included gradual development of unilateral weakness (N=4), non-fluent dysphasia (N=1), somnolence (N=1), and headaches (N=3). Neurologic deficits resolved within 30 minutes to 10 days. Transient cortical enhancement was confirmed on MRI in two patients, two had a normal brain MRI, and one had no MRI obtained. Two children had a single and three had multiple episodes over the next few months. Topiramate was successfully used in one and failed along with levetiracetam in another. Two children with protracted symptoms responded to high dose intravenous methyl prednisone for three days followed by 2 weeks of oral taper. Symptoms ultimately resolved in all patients. CONCLUSION: SMART syndrome is a rare disorder characterized by slow development of neurologic deficits with variable occurrence of abnormal cortical contrast enhancement. The use of anti-epileptics and/or steroids may improve symptoms and speed resolution.

NCMP-27. QUALITY OF LIFE IN LONG-TERM SURVIVORS OF PEDIATRIC CANCER: THE IMPACT OF HEARING LOSS

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BACKGROUND: Cisplatin chemotherapy causes irreversible hearing loss (HL) in approximately 50% of children and adolescents. We examined the association between cisplatin-induced HL and health-related quality of life (HRQOL) in a cohort of childhood cancer survivors. METHODS: Participants were ≤18 years at time of treatment, received a cumulative cisplatin dose ≥200 mg/m^2, and had at least one year between treatment completion and study enrollment. HL was graded using the International Society of Pediatric Oncology Ototoxicity Scale. HRQOL was assessed with self- and parent-reported versions of the Pediatric QOL Inventory (PedsQL). A questionnaire was developed for survivors and parents to assess the use of hearing technology, communication difficulties, and educational needs. Data were analyzed using linear regression and Fisher's exact test. RESULTS: Data from 66 patients (36 M/30 F) are summarized. The median age at diagnosis was 8.8 years (range 1 month-18 years), and the age range at the time of study enrollment was 3.6–35.1 years. The average time since treatment completion was 9 years (range 1.5–22 years). Diagnoses included medulloblastoma (39%), osteosarcoma (27%), neuroblastoma (14%), hepatoblastoma (8%) and other (12%). At the end of treatment 27% had no HL while 38% had mild HL and 35% had severe HL. Self- and parent-reported HRQOLs were strongly associated ($P < 0.0001$) and there were no differences in HRQOL among HL groups. However, survivors with severe HL more frequently reported speech and language delay ($p<.01$), learning disability ($p<.05$), limitation in activities ($p<.01$), and need for special education ($p<.01$). CONCLUSIONS: In this large cohort of long-term childhood cancer survivors, severe HL was significantly associated with communication and learning difficulties and need for educational supports, but not with HRQOL as measured by the PedsQL.

NCMP-28. PTPRZ1-MET SIGNALING PROMOTES GLIOMA PROGRESSION THROUGH STIMULATION THE TRANSFORMATION FROM M1 TO M2 MACROPHAGE

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INTRODUCTION: Tumor-associated macrophages (TAMs) have multiple functions in both inhibiting or promoting diversity tumors progression and correlated with increased intratumoral heterogeneity. PTPRZ1-MET (ZM) fusion has been implicated in the development of glioma and recently associated with an unfavorable prognosis for afflicted secondary glioblastoma (sGBM) patients, as well as temozolomide chemoresistance. The underlying mechanisms of ZM fusion and TAM in glioma still remain undefined. METHODS: SsGSEA is a rank-based method that computes an overexpression measure for a gene list of interest relative to all other genes in the genome. The ssGSEA scores for M1-type and M2-type macrophage scores are standardized across all tumor with known M1-type and M2-type macrophage properties in previous reports. The influence of ZM fusion on macrophage conversion in glioma was explored by performing gene set enrichment analysis and in vitro and in vivo experiments. An orthotopic xenograft model was established in this study. RESULTS: Here, we first show that the ssGSEA score of M2-type macrophage is upregulated in ZM fusion positively gliomas and M2-type macrophage score is highly associated with glioma patient overall survival. Further studies illustrated that the abundant macrophages populations and M1–M2 polarization of macrophage are tightly controlled processes of the hyper-activation of PTPRZ1-MET signaling in vitro and in orthotopic xenograft model. Meanwhile, our data also indicate that distinct transcriptional networks in brain-resident microglia and recruited bone-marrow-derived macrophages (BMDMs) are influenced by PTPRZ1-MET signaling pathway. CONCLUSION: These data indicate that PTPRZ1-MET signaling contribute to glioma malignant progression by recruiting macrophages and facilitating M2-type to M1-type macrophages conversion and therefore provide a novel therapeutic target for the treatment of sGBM patients.

NCMP-29. CEREBRAL EDEMA FROM RAPIDLY PROGRESSIVE METASTATIC CNS ATRT AND CHEMOTHERAPY INDUCED TUMOR LYSIS

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INTRODUCTION: Young pediatric patients with Central Nervous System (CNS) Atypical Teratoid Rhabdoid Tumor (ATRT) often present with metastasis and have extremely poor prognosis with the 2-year event-free survival of 11% or less. We report a case of metastatic CNS ATRT with literature review. RESULTS: A 22-month-old girl presented with subacute onset ataxia, vomiting, weight loss, headaches, motor and speech regression followed by acute onset seizures. MRI brain revealed a heterogeneously enhancing solid-cystic pineal mass causing hydrocephalus. Tumor biopsy confirmed ATRT, WHO grade IV, SMARCB1/ INI1 loss CSF cytology revealed M3 disease. Germline rhabdoid predisposition syndrome was ruled out. The primary tumor was resected. Immediate postoperative MRI was concerning for further metastasis of the tumor. Chemotherapy based on

Medical University of Vienna (MUV) protocol was begun but within 36 hours of doxorubicin, the patient succumbed to acute respiratory failure, hyponatremia, seizures and cardiac arrhythmia. CT head showed diffuse cerebral edema and early transtentorial herniation. A limited autopsy showed brain tissue studded with tumor cells. DISCUSSION: In our patient, the primary tumor was resected based on the reports of improved event-free survival in the setting of reduced tumor burden. MUV chemotherapy was started given its best-reported outcomes so far with the 5-year event-free survival of 89% in patients with M1-M3 CNS ATRT. Our patient may have suffered from doxorubicin-induced acute arrhythmia, rare but a known complication. In the light of rapidly metastasizing tumor, the possibility of doxorubicin-induced tumor lysis leading to acute cerebral edema cannot be excluded. Thus a combination of rapidly progressive metastatic tumor and rare chemotherapy complications may have led to the fatal outcome.

NCMP-30. OUTCOME OF CONCOMITANT CHEMORADIATION WITH TEMOZOLOMIDE FOLLOWED BY TEMOZOLOMIDE IN PATIENTS WITH GLIOBLASTOMA MULTIFORME

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BACKGROUND: Glioblastoma is the most common primary brain tumor in adults. The current standard of care is multimodal approach comprising of maximal safe surgical resection, post-operative radiation therapy (RT), and concurrent and adjuvant temozolomide (TMZ). To date, no published data are available for the outcome of this disease treated with standard of care in Pakistan. In this study results of concomitant chemoradiation with 5 days of temozolomide followed by temozolomide has been reported. OBJECTIVE: To determine Progression Free Survival (PFS) and Overall Survival (OS) in patient's receiving concomitant chemoradiation with 5 days of temozolomide followed by temozolomide in newly diagnosed GBM. METHODS: Patients with newly diagnosed glioblastoma were assigned to receive concomitant chemoradiation with temozolomide (75mg/m2) 5 days per week on the days of radiation. After a 4-week break, patients then received monthly temozolomide (150-200mg/m^2) 5-day schedule every 28 days. RESULTS: From June 2011 to June 2016, 58 patients with newly diagnosed GBM were included in the study. Median age was 54 years, 71% (n=41) were male, whereas 29% (n=17) were female. 57% (n=33) underwent subtotal resection, 33% (n=19) had gross total resection while 10% (n=6) underwent biopsy only. About 64% (n=37) completed standard of care concomitant chemoradiation followed by monthly TMZ.36 % (n=21) completed only concomitant chemoradiation and were lost to follow. At a median follow up of 1 year, median PFS and OS was 8 and 13 months respectively with chemoradiation followed by TMZ. In subgroup analysis median PFS and OS benefit was more in patients who underwent maximal safe resection as compared to biopsy. CONCLUSION: This study confirmed that PFS and OS benefit was more in patients who completed concomitant chemoradiation followed by monthly TMZ. OS benefit was almost similar with 5 days of TMZ as opposed with 7 days of TMZ as reported in international study.

NCMP-31. RISK OF INTRACRANIAL HEMORRHAGE (ICH) IN PATIENTS WITH HIGH-GRADE GLIOMA (HGG) ON THERAPEUTIC LOW MOLECULAR WEIGHT HEPARIN (LMWH)

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BACKGROUND: Venous thromboembolism (VTE) occurs in 20–30% of HGG patients. The potential increased risk of ICH with therapeutic anticoagulation complicates VTE treatment. Recent retrospective study reported a 3-fold increased risk of ICH with therapeutic anticoagulation. Our study examines whether the administration of LMWH for VTE treatment is associated with increased risk of ICH in HGG patients. METHODS: We performed a retrospective cohort study of HGG patients from 1/2005–8/2016. Patients in the LMWH group initiated treatment after VTE diagnosis. Blinded review of neuroimaging for ICH was performed. Patient characteristics were summarized using frequency for categorical variables and mean for continuous variables. For analysis of the primary end point, estimates of cumulative incidence (CI) of ICH were calculated using competing risk analysis with death as competing risk; significance testing was performed using the Gray's test. RESULTS: A total of 174 patients (100 men) were included, 88 (51%) in the LMWH group and 86 (49%) in non-LMWH group. Within the non-LMWH group, 22 (26%) patients developed VTE but were not treated with anticoagulation, while 64 (74%) patients did not develop VTE. A total of 34 ICH were recorded: 19 (56%) in LMWH group, 3 (9%) in non-LMWH with VTE, and 12 (35%) in patients without VTE.No significant difference was observed in the 1-year CI of ICH in the LMWH cohort and non-LMWH with VTE group (17% versus 9%; Gray's test, p=0.36). Among patients without VTE, the 1-year CI of ICH was 13%; direct comparison to LMWH group was not performed due to different starting time point of events. CONCLUSIONS: Contrary to recent studies, our data do not suggest that therapeutic LMWH is associated with significantly increased risk of ICH in HGG patients.

NCMP-32. OUTCOMES AND PREDICTIVE FACTORS OF RECRANIOTOMY FOR SEVERE COMPLICATIONS FOLLOWING SELECTIVE CRANIOTOMY FOR INTRACRANIAL TUMORS

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OBJECTIVE: The outcomes of recraniotomies for severe complications following selective craniotomies for intracranial tumors were rarely reported by far. Hence, the objective of this study was to analyze the outcomes and predictive factors of unscheduled recraniotomies. METHODS: Patients undergoing recraniotomies for complications after selective craniotomies for intracranial tumors from Jan 2014 to Dec 2017 were collected. Primary outcome was KPS (Karnofsky Performance Status) score at discharge. Patients were divided into three groups according to this KPS score: good KPS group with KPS≥70, poor KPS group with KPS≤10, and others were moderate KPS group. Predictive factors included demographics, imaging findings and characteristics of two craniotomies. RESULTS: There were 109 patients included with 39 patients in good KPS group, 39 in moderate KPS group, and 31 in poor KPS group. The rate of poor KPS outcome was 28.4%. Univariate and multivariate regression analysis showed tumor diameter (odds ratio 0.589, P=0.016), reoperative blood loss (odds ratio 0.001, P=0.021), and time interval between two craniotomies (odds ratio 13.680, P=0.000) were positively associated with poor KPS. In contrast, GCS score before recraniotomies (odds ratio -0.713, P=0.004) were positively associated with good KPS. CONCLUSIONS: The major outcome of recraniotomies was good and moderate KPS at discharge. Tumor diameter, reoperative blood loss, and time interval between two craniotomies were positive predictive factors for poor KPS outcome. GCS score before recraniotomies was positive factors for good KPS outcome.

PEDIATRIC CLINICAL TRIALS

PDCT-02. COMBINED INHIBITION OF MTORC1/C2 AND MEK PATHWAY IS SYNERGISTIC IN PRECLINICAL TESTING OF PEDIATRIC LOW-GRADE GLIOMA INCLUDING A NOVEL PATIENT-DERIVED NF1 PILOCYTIC ASTROCYTOMA CELL LINE

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Pediatric low-grade glioma (PLGG) is the most common brain tumor of childhood. We and others have identified mTOR and MEK-pathway activation in PLGG. The dual mTORC1/2-inhibitor, TAK228, and the FDA approved MEK-inhibitor, trametinib, are promising candidates for targeted PLGG therapy. We treated five different patient-derived PLGG cell models with TAK228 and/or trametinib: JHH_NF1_PA1 (NF1 mutation), BT66_SV40/hTERT (BRAF:KIAA1549 fusion), BT40 (BRAFV600E) Res186 (PTEN deletion) and Res259 (PDGFRα amplification and CDKN2A deletion). Treatment with TAK228 or trametinib reduces cell proliferation in a dose and time depended manner investigated via MTS-assay. Both drugs exert a synergistic effect at 5-20nM in JHH_NF1_PA1, Res186, and Res259 cells as calculated by the method of Chou-Talay. BT66_SV40/hTERT cells have a 70% reduction in cell growth with 10nM TAK228 ($p < 0.001$ by ANOVA) but not in combination with trametinib. In all cell lines trametinib treatment leads to a pERK inactivation at low nM levels. TAK228 treatment leads to inactivation of mTORC1 and mTORC2. Apoptosis induction was examined through cleaved PARP via western blot and CC-3 via immunocytochemistry. The combination of TAK228 and trametinib increased apoptosis by up to 127% ($p < 0.001$) in Res186, Res259, and JHH_NF1_PA1 cells. We tested trametinib and TAK228 against the BRAFV600E mutant BT40 patient derived xenograft in immunodeficient mice. In combination TAK228 and trametinib decreased significantly BT40 tumor growth compared to vehicle or either agent alone, ($p < 0.01$ by ANOVA). Striking was the reduced vascularization of the tumor tissue after combination treatment compare the vehicle control and single agent treatment. Vascularization differences will be evaluated with immunohistochemistry staining for PDGFα and PECAM-1. Our results show that PLGG-derived cell lines are sensitive to TORC1/2 kinase inhibition and MEK inhibition. Further, our *in vivo* experimentation provides the first strong rationale for combination therapy of these agents in aggressive PLGG.

PDCT-03. A PHASE II TRIAL OF POLY-ICLC IN THE MANAGEMENT OF RECURRENT OR PROGRESSIVE PEDIATRIC LOW GRADE GLIOMAS. RESULTS FOR THE NEUROFIBROMATOSIS 1 GROUP. (NCT01188096)

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BACKGROUND: Polyinosinic-Polycytidylic acid stabilized with polylysine and carboxymethylcellulose (poly-ICLC) is a double stranded RNA that acts as a ligand for the Toll Receptor 3 (TLR3) and results in a broad host defense stimulation, including T-cell and natural killer cell activation and cytokine release (interferons alpha, beta, and gamma, interleukins, corticosteroids, and TNF). The goal of this trial is to determine the efficacy of this novel immunotherapy against refractory pediatric low-grade glioma (LGG). Here we present the results for the neurofibromatosis group. METHODS: Poly-ICLC 20mcg/kg/dose IM was self-administered twice a week at home for up to 24 cycles. Each cycle is 28 days. Endpoints include objective tumor response rate, progression-free survival (PFS), and rate of stabilization/improvement in visual examination in those with optic pathway glioma. RESULTS: Twenty three patients were enrolled, six with NF-1. Of NF1 patients (3 males, 3 females), five were evaluable, ages 1–16 years. Tumor location: 4 optic pathway, one thalamic, one cervico-medullary junction. 5/6 had received more than 2 prior regimens (range 1–7). Responses (PR+SD) were observed in 4/5 (80%), with 2 SD having a decrease in tumor size by 35% and 10%, one of whom had significant improvement on visual field testing, and 2 PR having decrease in tumor size by 50% and 55%. Only low grade toxicities were observed with poly-ICLC, including erythema and pain at site of injection, fever, myalgias, and ALT elevation. One intra-tumoral hemorrhage occurred with complete resolution with medical management alone. All patients are alive. CONCLUSION: Poly-ICLC is extremely well tolerated in the NF-1 group and the single agent response rate in this small NF1 cohort is encouraging. Expanded phase II testing of poly-ICLC in the NF-1 cohort is planned.

PDCT-04. PHASE 1 TRIAL OF WEE1 KINASE INHIBITOR AZD1775 COMBINED WITH RADIATION THERAPY FOR CHILDREN WITH NEWLY DIAGNOSED DIFFUSE INTRINSIC PONTINE GLIOMA: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP PHASE 1 PILOT CONSORTIUM (ADVL1217)

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OBJECTIVES: Children with diffuse intrinsic pontine glioma (DIPG) continue to have a dismal outcome and median survival remains stagnant at 9 months for decades. AZD1775 is an orally available inhibitor of Wee1 kinase, a key G2-M checkpoint regulator that has been shown to cross the blood brain barrier and has demonstrated efficacy in preclinical DIPG studies. METHODS: AZD1775 was administered orally in newly diagnosed children with DIPG, only on days of radiation therapy. The protocol assessed 6 dose levels starting from 50 mg/m^2/dose and escalated up to 200mg/m^2/dose. Dose escalation occurred first by the number of days on which AZD1775 was administered and then by an increase of the actual dose. The entire length of radiation therapy constituted the dose limiting toxicity period. Correlative studies included pharmacokinetic (PK) analyses as well as determination of expression of p-CDC2, p-HH3 and g-H2AX in peripheral blood mononuclear cells (PBMC). In the late breaking abstract we will present results of this phase 1 study including PK analyses as well as results of the correlative studies. Results of this study will lay the groundwork for subsequent clinical trials using AZD1775 in pediatric brain tumors.

PDCT-05. FEASIBILITY OF PROSPECTIVE WHOLE-EXOME SEQUENCING (WES) IN PEDIATRIC MEDULLOBLASTOMA PATIENTS ON THE HEAD START 4 PROTOCOL

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BACKGROUND: Medulloblastoma is the most common brain tumor in early childhood. Molecular profiling of medulloblastomas has resulted in a classification system including at least 4 major subtypes of disease, defining prognosis in patients treated largely on traditional chemotherapy and irradiation-based protocols. The Head Start Consortium has developed treatment protocols designed to avoid irradiation and its debilitating long-term side effects. The first three Head Start trials showed promising results; however, prospective genetic data are still lacking to evaluate whether molecular alterations can predict patient response to irradiation-avoiding treatment strategies. The Head Start 4 Protocol is a multi-national trial that incorporates non-mandatory, prospective tumor and blood collection for analysis in an attempt to address this question. Currently, 100% of patients enrolled to date have submitted samples for WES. METHODS: Paired blood and tumor samples (FFPE) from the first 10 medulloblastoma patients had WES (SureSelect Human All Exon V6+COSMIC) performed utilizing an Illumina Hiseq 4000. Data were processed using varscan v2.4.1 somatic command on tumor/normal pairs and annotated using Variant Effect Predictor from ensembl. Results were filtered at a threshold of 20 read depth or 40% variant allele frequency. Low-impact mutations were removed. Mutation profiles were compared with pediatric medulloblastoma gene mutation data from the PedcBioPortal and PeCan databases. RESULTS: A total of 903 mutation events were identified across 10 patients with each patient demonstrating mutations in one or more genes previously reported to be mutated in medulloblastoma. Two samples harbored SUFU mutations similar to those reported previously. CONCLUSIONS: These data show the feasibility of prospective high-dimensional mutational analysis using non-mandatory tissue submissions for pediatric medulloblastoma patients enrolled on the Head Start 4 Protocol. Copy-number and methylation analysis is ongoing. FUNDING: Thrasher Research Fund (JF, AC). R01CA108633, R01CA169368, RC2CA148190, U10CA180850-01 (NCI), Brain Tumor Funders Collaborative Grant, and OSUCCC (AC).

PDCT-06. PHASE 1 STUDY OF ONC201 IN PEDIATRIC PATIENTS WITH H3 K27M-MUTANT HIGH GRADE GLIOMA OR NEWLY DIAGNOSED DIPG

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The imipridone ONC201 is the first selective antagonist of DRD2 for clinical oncology. Several Phase 1, Phase 1/2 and Phase 2 studies in patients with advanced cancers have established the single agent recommended Phase 2 dose (RP2D) of 625mg ONC201 administered orally once a week in adults. ONC201 induces p53-independent apoptosis in newly diagnosed and recurrent high-grade glioma in vitro, ex vivo and in vivo. Furthermore, radiographic regressions in adult recurrent H3 K27M-mutant glioblastoma patients in response to single agent ONC201 have been reported. Based on this adult experience and complementary preclinical results demonstrating the increased susceptibility of H3 K27M-mutant gliomas to ONC201, we initiated the first Phase 1 pediatric clinical trial of ONC201 January 30, 2018. This trial will determine the safety and RP2D of ONC201 in pediatric post-radiation H3 K27M-mutant glioma patients as a single agent and in newly diagnosed diffuse intrinsic pontine glioma (DIPG) patients in combination with radiation (NCT03416530). Patients without known H3 K27M-mutation status by a CLIA-lab can enroll with commitment to post-term biopsy. This is a multicenter, open-label, 2 arm, dose-escalation and dose-expansion study. Ten children with H3 K27M-mutant gliomas ages 5–18 years have been treated post-radiation: 3 at dose level 1; 3 at dose level 2; 4 as part of the dose expansion cohort on dose level 2. Patients have received 2–12 doses (median= 5). The ONC201 has been tolerated very well. Grade III/IV events include: decreased neutrophil count grade III (n=1) spontaneously resolved without dose modification and elevated AST grade III (n=1) returned to grade II after holding 1 dose. Additional safety data as well as pharmacokinetics, pharmacodynamics, and progression-free survival results from this trial will be reported.

PDCT-07. FEASIBILITY TRIAL OF TTFIELDS (TUMOR TREATING FIELDS) FOR CHILDREN WITH RECURRENT OR PROGRESSIVE SUPRATENTORIAL HIGH-GRADE GLIOMA (HGG) AND EPENDYMOMA: A PEDIATRIC BRAIN TUMOR CONSORTIUM STUDY: PBTC-048

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BACKGROUND: Children with recurrent or progressive pediatric CNS tumors have poor prognoses. Optune delivers TTFields, an anti-mitotic FDA-approved cancer therapy, for adult patients with newly diagnosed and recurrent supratentorial GBM. Methods: This multicenter trial [NCT03033992] examines the feasibility and device-related toxicity of TTFields in 20 children aged 5–21 years with recurrent supratentorial HGG and ependymoma. Secondary objectives include: response rate, event-free survival, compliance and QoL. Feasibility in this trial is defined as the ability of pediatric subjects to wear Optune ≥18 hours/day for at least 23/28 days of cycle one following a 7-day learning period. Treatment may continue up to 26 cycles based on observed benefit and safety. QOL instruments include PROMIS and Neuro-QOL. Following enrollment of the 11th evaluable subject, the study will be temporarily suspended to share interim feasibility and safety data with the FDA. RESULTS: This planned interim analysis included 11 patients (7 males/4 females) with supratentorial tumors: ten HGG and one ependymoma. Median age was 14.2 (6.4–21.3) years. Ten patients were evaluable (1 Progressive Disease) during the feasibility period) and 4 remained on study through 4 cycles and one patient is currently on Cycle 14. One grade 5 intracranial hemorrhage not associated with the device and no grade IV toxicities occurred, 3 patients had seizures (grade 1–3), fatigue, scalp pain, localized rash, and headache (none greater than grade 3). Of the 10 evaluable patients, 7 satisfied the feasibility criteria for the Optune therapy, which is above the prespecified threshold of at least 6/11. CONCLUSION: This is the first prospective safety and feasibility trial for Optune in children with recurrent supratentorial HGG and ependymoma. Preliminary results indicate feasibility with minimal toxicity. Accrual to the study is ongoing. An amendment is in progress adding a stratum for newly diagnosed diffuse intrinsic pontine glioma (DIPG) patients.

PDCT-08. TRACKING THE T CELL REPERTOIRE AFTER ADOPTIVE CELL THERAPY IN PEDIATRIC PATIENTS WITH RECURRENT MEDULLOBLASTOMA

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BACKGROUND: Adoptive cellular therapy (ACT) using transfer of tumor-specific lymphocytes is a promising way to treat tumor patients. Tumor-specific T cells can lead to tumor regression in some cancer patients who do not respond to other therapies; however, the ability to track these cells after transfer has remained limited. In this study, high-throughput deep sequencing was used to track the T cells after in vivo infusion in pediatric patients with central PNETs. METHODS: Bulk peripheral blood mononuclear cells (PBMCs) are stimulated ex vivo using autologous DCs loaded with total tumor RNA. We used high-throughput T cell receptor sequencing to identify and track clones and their frequency in patients T cell isolates collected prior to adoptive cellular therapy and weekly for one month and then monthly following immunotherapy treatment. cDNA was generated with addition of a common adapter at 5 end of cDNA using RACE technology. RESULTS: A broad range of diversity was observed with 12772 to 33709 individual clones being identified in the different samples after ex vivo expansion. The most prevalent clone represented between 0.61 and 20% of the population depending on the patient. The patients with prolonged progression-free and overall survival maintained high frequency ex vivo expanded clonotypes for many months post infusion. We observed in these ex vivo-expanded samples significantly higher expression level of markers associated with long-lived memory T cells (CD27 and CD127) compared to short overall survival patients. In most patients, the clonal hierarchies in the ACT product did not correlate with the peak clonotype hierarchys post infusion suggesting considerable restructuring of T cell repertoire once product went in vivo. CONCLUSIONS: TCR deep sequencing can be used to quantitatively track ACT-derived T cells post infusion. Monitoring of TCR repertoire dynamics during ACT may provide an early biomarker for treatment response and clinical outcomes.

PDCT-09. PHASE 1/2 STUDY OF DSP-7888 IN PEDIATRIC PATIENTS WITH MALIGNANT GLIOMA

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BACKGROUND: DSP-7888 is an experimental cancer vaccine containing peptides that induce WT1-specific CTLs and helper T cells. A phase 1/2 study in pediatric patients (pts) with malignant glioma was conducted to evaluate the efficacy and safety. METHODS: Diffuse intrinsic pontine glioma (DIPG), glioblastoma (GBM) and other high grade glioma (HGG) pts for whom standard therapy failed or who have no available standard therapies were eligible. Pts received five-times DSP-7888 3.5 mg/body via intradermal injection weekly followed by biweekly administration. Response was assessed with MRIs via modified RANO criteria. Recommended dose was determined based on the traditional 3 + 3 design. RESULTS: Four pts were enrolled in phase 1 dose finding part (3.5 mg/body). One patient was replaced due to primary disease progression and the three pts completed DLT evaluation and no DLTs were observed. Therefore, recommended dose was determined as 3.5 mg/body. In a whole study, 18 pts (11 DIPG, 5 GBM and 2 HGG) were enrolled. There was no dose-limiting or unexpected toxicity. Most common treatment related adverse events was controllable injection site reaction only. Disease control (CR+PR+SD) was observed in 7 of 18 pts, with 1 PR and 6 SD. Median OS from initial dose in DIPG exceeded 5 months. Three out of 5 GBM pts are on survival more than 11 months. WT1 specific CTLs induction were observed in several pts. CONCLUSIONS: DSP-7888 is well tolerated at 3.5 mg/body for pts with pediatric malignant glioma. Survival results in DIPG pts exceeded in comparison with historical controls. Moreover, some GBM pts shows encouraging sign of long survival.

PDCT-11. SURVEILLANCE DATA DEMONSTRATES THE TOLERABILITY OF TUMOR TREATING FIELDS IN PEDIATRIC GLIOMA PATIENTS

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INTRODUCTION: There are few treatment options for pediatric patients with high grade gliomas Adult patients with newly diagnosed or recurrent glioblastoma multiforme have the option of treatment with tumor treating fields (TTFields), which have shown efficacy and safety in Phase 3 randomized trials. TTFields are a noninvasive anti-mitotic therapy administered via transducer arrays strategically arranged on the shaved scalp. TTFields are not approved for the treatment of high grade glioma in pediatric patients. Post-marketing surveillance data were collected for pediatric glioma patients receiving TTFields. Compiled safety data are presented for patients <18 years receiving TTFields for the treatment of glioma. METHODS: A review of the clinical information for pediatric patients (<18 years of age) treated with TTFields in the United States and Europe identified 30 patients. The safety data obtained from post-market surveillance in all 30 patients were analyzed to identify adverse events (AEs) based on the MedDRA body system (system organ class) preferred terms. RESULTS: Sixteen (53%) pediatric patients experienced at least 1 AE while receiving TTFields treatment. Patients reported general AEs include: electric sensations in 10% (3/30); fatigue in 10% (3/30), heat sensation in 10% (3/30) and 17% reported pain (5/30) in any location. Nervous system AEs included headache (10%), seizure (7%) and balance disorder (7%). The most common AE were skin reactions that were reported by 7 patients (23%). One patient reported hyperhidrosis (3%). CONCLUSIONS: There were no unexpected AEs associated with TTFields in pediatric patients. The most frequently reported AEs were skin reactions likely related to the placement of TTFields transducers on the scalp; incidence was comparable to the rate of skin reactions reported in adult GBM patients. The tolerability of TTFields in pediatric patients from this post-marketing surveillance data support further investigations to assess efficacy and safety of TTFields in pediatric glioma patients.

PDCT-12. A PHASE I TRIAL OF TUMOR TREATING FIELDS WITH AND WITHOUT CONCOMITANT TEMOZOLOMIDE AND BEVACIZUMAB IN PEDIATRIC PATIENTS WITH HIGH-GRADE GLIOMA AND EPENDYMOMA

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High-grade gliomas (HGG) comprise 8–12% of brain tumors in children and include anaplastic astrocytoma or glioblastoma multiforme (GBM) pathology. Ependymoma is the third most common pediatric brain tumor accounting for 5–10% of all diagnoses. Treatment for these tumors involves surgical resection, adjuvant radiation and chemotherapy. The prognosis for high-grade glioma and recurrent ependymoma is poor and new therapies must be investigated to improve clinical outcomes for these patient populations. Tumor treating Fields (TTFs) is a non-invasive antimitotic treatment comprised of low-intensity alternating electric currents delivered through the Optune device approved for adults with GBM. This phase 1 study [NCT03128047] will include 2 cohorts of pediatric patients (N= 6–12), age ≥1 and <18 years, with histologically-confirmed supratentorial newly-diagnosed or recurrent HGG and recurrent ependymoma. This study will have two safety endpoints to assess the safety and tolerability of TTFs for the treatment of pediatric HGG and ependymoma both alone and in combination with temozolomide (TMZ) and bevacizumab (BEV). Cohort 1 patients with recurrent HGG or ependymoma will receive TTFs (200 kHz 18 hours/day). Cohort 2 patients with newly-diagnosed or recurrent HGG or recurrent ependymoma will receive TTFs plus BEV 10 mg/kg/dose and TMZ 200 mg/m2/day. Safety endpoints will be determined by a standard 3 + 3 phase I study design based on the incidence and severity of adverse events and toxicities (CTCAE V4). There are no primary efficacy endpoints for this study. Patients will be followed to assess their progression-free and overall survival as the efficacy data gained from this study may be useful in designing future phase II/III investigations. The sample size will be determined by the number of observed severe adverse events related to therapy. A minimum of two and a maximum of twelve patients will be needed to complete this study.

PDCT-13. PINEOBLASTOMA IN CHILDREN: THE HEAD START EXPERIENCE

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BACKGROUND: Pineoblastoma is a rare malignant pineal region tumor, the optimal management of which in young children remains unclear. METHODS: We report the outcomes of 23 children with pineoblastoma prospectively enrolled on three sequential Head Start (HS) trials between 1990 and 2009. The HS treatment strategy included maximal surgical resection followed by five cycles of intensive induction chemotherapy and consolidation with marrow-ablative chemotherapy and autologous hematopoietic cell rescue (HDCx/AuHCR). Irradiation following recovery from consolidation was reserved for children over six years old or with residual tumor. RESULTS: Data on 23 children with pineoblastoma including two with trilateral retinoblastoma aged 0.44–5.72 (median 3.12) years were analyzed. Median overall survival (OS) was 12 months (95% CI: 7.6–29.7 months). The 3-year progression-free survival (PFS) and OS were 14.5% (95% CI: 5.1–41.2%) and 17.4% (95% CI: 7.1–42.4%), respectively. Three patients were long-term survivors beyond 5 years. Eight patients experienced progressive disease (PD) during induction chemotherapy, six following the fourth and fifth cycles. Ten patients proceeded to consolidation with HDCx/AuHCR; eight experienced PD post-consolidation. Seven patients received craniospinal irradiation with boost(s) (median dose 20.7 (18–36) Gy), three patients as adjuvant therapy and four upon progression/recurrence. Favorable prognostic factors were administration of radiotherapy (hazard ratio (HR) for OS=0.30 (0.11–0.86), p<0.025), and undergoing HDCx/AuHCR (HR for OS=0.40 (0.16–0.99), p<0.047). There were no statistically significant associations between age, metastasis and extent of surgical resection and OS. CONCLUSIONS: Radiotherapy and HDCx/AuHCR were the sole driving factors for positive impact on survival. No benefit of high-dose methotrexate during induction was demonstrable. The high PD rate during later induction cycles as well as following consolidation chemotherapy warrant consideration of (1) fewer induction cycles prior to consolidation, (b) intensification of consolidation with multiple cycles of marrow-ablative chemotherapy and (c) judicious post-consolidation irradiation, in order to improve outcomes.

PEDIATRIC TUMORS

PDTM-01. GERMLINE GENETIC PREDISPOSITION TO PEDIATRIC GLIOMA

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INTRODUCTION: Pediatric gliomas constitute a majority of pediatric brain tumors and intermediate to high grade gliomas have high mortality. While some advances have been made over recent years with regards to pediatric glioma etiology, genetic predisposition has not been investigated thoroughly. In this study, the role of rare germline variants on risk of pediatric glioma was evaluated in 322 astrocytoma patients (predominantly grade II and III) using whole-exome sequencing and detailed evaluation of 162 known cancer-related genes. METHODS: DNA samples were extracted from neonatal dried bloodspots from 322 pediatric glioma patients born and diagnosed in California, and sequenced using the Personalis ACE whole-exome sequencing platform. Exome alignment and variant calling was performed using Samtools and GATK 3.8. SnpEff and Combined Annotation Dependent Depletion (CADD) tools were used for variant annotation. Only variants that were not present in the 1000 Genomes dataset, had allele frequency smaller than 0.01% in the Exome Aggregation Consortium (ExAC) database, a CADD score greater than 20, and a moderate or high variant effect impact were included to identify rare putatively deleterious variants. RESULTS: All samples passed quality control and the mean read depth was 41.4. Seventy-one (22.0%) samples harbored a mutation that was potentially deleterious in at least one of the 162 cancer-related genes. The most commonly affected gene encodes a specific receptor tyrosine kinase (RTK) (28 patients, 8.7%), followed by a DNA polymerase enzyme(5 patients), and a specific mismatch repair protein(5 patients). Alterations of the tyrosine kinase protein may impact cell growth, mutation, and maturation of nerve cells. CONCLUSION: This study supports a role for rare germline mutations in the etiology of pediatric glioma and implicates RTK biology in glioma predisposition. These findings have translational implications for risk prediction and targeted therapy.

PDTM-02. STRESS GRANULES ARE INDUCED BY OXIDATIVE STRESS IN PEDIATRIC BRAIN TUMORS AND PREDICT POOR OUTCOME

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BACKGROUND/OBJECTIVES: Brain tumors represent the most common and aggressive pediatric cancer type, underscoring a dire need for novel therapeutic approaches. Tumors are continually exposed to acute changes in their microenvironment, including oxidative stress. To overcome acute stress, cells form stress granules (SGs), clusters of RNA and RNA-binding proteins (RBPs) that rapidly alter the cellular mRNA translation landscape. Preliminary data indicate that pharmacological inhibition of SG formation blocks the antioxidant response of the transcription factor NRF2 (NFE2L2), impairing pediatric sarcoma invasive and metastatic capacity. We therefore set out to determine if pediatric brain tumors rely on SGs to overcome oxidative stress, and if targeting SGs could represent a therapeutic approach for these tumors. METHODS: We analyzed public databases for links between mRNA expression of the RBP G3BP1 and NFE2L2. Immunohistochemistry (IHC) for G3BP1, NRF2 and oxidative stress markers (4HNE) was performed on atypical theratoid rabdoid tumor (AT/RT), pediatric glioblastoma (pGBM) and ependymoma (EPN) tissue microarrays. AT/RT, pGBM and EPN cell lines were treated with $NaAsO_2$ and H_2O_2 to induce oxidative stress and SG presence was determined by ImmunoFluorescence for the RBPs G3BP1 and TIA-1. RESULTS: G3BP1 and NFE2L2 expression positively correlates in several pediatric tumor cohorts, including AT/RT, pGBM and EPN (p<0.01). IHC not only confirmed mRNA results, but revealed that G3BP1 over-expression is linked to higher WHO grade and recurrent disease in EPN. High G3BP1 levels are also predictive of poor outcome in pGBM (p<0.05). Finally, oxidative stress induces the RBPs G3BP1 and TIA-1 to form SGs *in vitro*. CONCLUSIONS: SGs represent an important mediator for the adaptive response of pediatric brain tumors to acute oxidative stress. Inhibiting SG formation might therefore constitute a therapeutic approach for AT/RT, pGBM and EPN. Future studies will aim at confirming the efficacy of drugs that inhibit SG formation in pediatric brain tumors.

PDTM-03. CREDENTIALING NOVEL PEDIATRIC GLIOMA MODELS

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We have developed a simple and generalizable in vivo method for brain tumor modeling, mosaic analysis by dual recombinase-mediated cassette exchange (MADR). MADR allows for stable labeling of mutant cells expressing transgenic elements from a precisely-defined chromosomal locus. We have demonstrated the power and versatility of MADR by creating novel glioma models with mixed, reporter-defined zygosity, or with "personalized" H3.3-containing driver mutation signatures from pediatric glioma--each manipulation altering the spatiotemporal profile of resulting tumors. Further we have generated ependymoma models by employing patient-derived fusion driver mutations. Notably, each model displays divergent spatiotemporal tumor expansion profiles and cellular phenotypes. Now, we use single-cell RNA-seq to compare these models to their cells of mutation and to human datasets to elucidate the fundamental transcriptional programs and markers within and across these tumor types. This investigation will assess and credential these models against their clinical counterparts by scrutinizing the resulting datasets and validating their clinical relevance as pre-clinical models for therapeutic discovery and testing.

PDTM-04. THERAPEUTIC MODULATION OF CHOLESTEROL HOMEOSTASIS IN DIPG THROUGH MASSIVE GENERATION OF 24,25-EPOXYCHOLESTEROL

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Diffuse intrinsic pontine glioma (DIPG) remains a uniformly fatal childhood tumor for which novel pharmacological treatments are desperately needed. In recent work, we performed a small molecule screen in a pre-clinical DIPG model, identifying MI-2 as the top 'hit', showing anti-tumor activity *in vitro* and *in vivo*. MI-2 was developed as an inhibitor of the protein menin, an epigenetic regulator which has oncogenic activity in acute leukemia. Here, we characterize the mechanism of action of MI-2 in DIPG. We generated menin knockout patient-derived DIPG cell-lines using CRISPR/Cas9 and observed that they retained sensitivity to MI-2 at nanomolar concentrations (comparable to wild-type parental cells), suggesting a menin-independent mechanism of action in DIPG. Transcriptome analysis of DIPG cells after MI-2 treatment revealed significant upregulation of gene targets of LXR (liver X receptor), a ligand-activated transcription factor which promotes cholesterol efflux to reduce cellular cholesterol. Consistent with a cholesterol depleting activity of MI-2, we showed markedly reduced cholesterol in DIPG cells following MI-2 treatment, and rescue of MI-2 induced cell-death by provision of exogenous cholesterol. To investigate how LXR is activated, we performed LC-MS analysis of major sterols species in MI-2 treated cells, and observed massive upregulation of 24,25-epoxycholesterol, an oxysterol known to be a potent endogenous activator of LXR. These findings characterize MI-2 as a novel cholesterol depleting agent, acting via an epoxycholesterol-LXR axis to promote cholesterol efflux from tumor cells. Our work suggests DIPG may be uniquely sensitive to perturbations in cholesterol homeostasis and implicates LXR activation as a potential therapeutic strategy in DIPG.

PDTM-05. RADIATION DNA DAMAGE REPAIR INHIBITION BY GSK-J4 INDUCED CHROMATIN COMPACTION IN DIPG

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INTRODUCTION: Focal radiation therapy has long been and remains the only treatment option for diffuse intrinsic pontine glioma (DIPG). However, all patients show evidence of disease progression within months of completing radiation therapy (RT). Since chemotherapy does not provide significant outcome improvement, it is crucial to find a suitable radiosensitizer. Our research has shown that the JMJD3 demethylase inhibitor, GSK-J4, exerts potent anti-tumor activity on DIPG cells while restoring methylation. Our aim is to We hypothesized that GSK-J4 may inhibit radiation-induced DNA repair, making it a potential radiosensitizer. METHODS: RNA sequencing (RNA-Seq) was used to analyze gene expression changes by GSK-J4 in DIPG cells. ATAC-seq was conducted to determine chromatin accessibility in DIPG cells treated with GSK-J4. We evaluated DNA damage repair using DNA repair assay and immunocytochemistry of DSB markers γH2AX and 53BP1. Western blotting and quantitative PCR (qPCR) were conducted to evaluate differential expression of mRNA and proteins involved in DNA DSB repair. *In vivo* response to radiation monotherapy and combination of RT + GSK-J4 were measured by animal survival studies. RESULTS: RNA-Seq and qPCR show that GSK-J4 significantly reduces DNA DSB repair genes in DIPG cells. ATAC-seq results reveal that GSK-J4 modifies DNA accessibility to regulate expression of DNA repair genes. Immunocytochemistry results support that GSK- J4 sustains high levels of γH2AX and 53BP1 in irradiated DIPG cells, thereby inhibiting DNA DSB repair. DNA repair assay demonstrate that GSK-J4 inhibits DNA damage repair via the homologous recombination pathway. Western blotting revealed that GSK-J4 causes a sustained level of phosphorylated Rad50 and gH2AX in irradiated DIPG cells. *In vivo* studies revealed increased survival of animals treated with combination therapy compared to monotherapy. These results highlight GSK-J4 as a potential radiosensitizer in DIPG treatment.

PDTM-06. ALK AMPLIFICATION AND REARRANGEMENTS ARE RECURRENT TARGETABLE EVENTS IN GLIOBLASTOMA

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Anaplastic Lymphoma Kinase *(ALK)* expression, rearrangements, and single nucleotide variants have been reported in several brain tumor types, but the significance and function of each aberration in adults and children have not been clearly established. To determine the degree to which *ALK* represents a relevant therapeutic target in gliomas, we first examined *ALK* expression using immunohistochemistry (IHC) in a panel of 148 adult GBM and 49 pediatric GBM/high grade gliomas. We identified high *ALK* expression was most frequent in pediatric gliomas (32%, 16/49, IHC score 2+ or 3+). Copy arrays/sequencing and FISH for the *ALK* locus revealed high level *ALK* amplification in 31% of *ALK*-expressing cases (5/16) but was a rare event in gliomas overall. Whole-genome sequencing and RNA sequencing identified novel and recurrent *PPP1CB-ALK* fusions in 43% of *ALK*-expressing pediatric GBM (7/16), suggesting IHC may be an efficient means of screening for *ALK* aberrations as in the identification of *EML4-ALK* lung cancer. All *ALK*- amplified cases harbored *PPP1CB-ALK* fusion but 2 *PPP1CB-ALK* cases were copy neutral. The phosphatase PPP1CB was fused in-frame to *ALK* at exon 20 with preservation of the *ALK* kinase domain and predicted to activate via the same mechanism as other *ALK* rearranged cancers. *ALK* fusion proteins promoted cell proliferation and constitutive kinase activity and upregulated STAT and AKT signaling pathways. However, expression of novel *ALK* missense mutations in NSCs did not promote proliferation. Administration of *ALK* inhibitor Crizotinib reduced tumor growth in a PDX GBM from a patient with a novel *ALK* fusion. This work validates amplification and rearrangement of *ALK* as a highly recurrent driver event and therapeutic target in GBM, particularly in young children where it may be the sole driver event.

PDTM-07. DETECTION OF IDH1 R132H MOSAICISM IN ANAPLASTIC ASTROCYTOMA PATIENTS

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Anaplastic astrocytomas are aggressive cancers of glial cells that present poor prognosis and high recurrence. Heterozygous mutations in IDH1 and IDH2 genes are common in the patients with anaplastic astrocytomas and glioblastomas. In the majority of cases, if not all, the IDH mutations seen in patients with brain tumors have been heterozygous somatic mutations. Patients with Ollier disease and Maffucci syndrome, which are rare enchondromatosis syndromes, have been shown to carry mosaic mutations of IDH1 or IDH2 in non-neoplastic tissues but no patients with anaplastic astrocytoma have been shown to carry such mosaicism. Here, we present two siblings with high grade astrocytoma with IDH1 R132H mutation. Our analysis of IDH1 R132H mutations in the siblings' tumors and non-neoplastic tissues, including healthy regions of the brain, cheek cells and primary teeth has identified IDH1 R132H mosaicism, using digital PCR system. IDH1 R132H mutant allelic frequencies ranging from 0.01% to 0.64% were detected in teeth, cheek cells and non-tumorous brain tissue. The findings were confirmed by next-generation sequencing with similar single nucleotide variation frequencies. Our study demonstrated an example of IDH1 R132H mosaicism in anaplastic astrocytoma patients, which could have gone unnoticed by traditional sequencing technologies.

PDTM-08. ROLE OF miR-212 AS A TUMOR SUPPRESSOR GENE IN NON-SHH/WNT MEDULLOBLASTOMA

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Medulloblastoma (MB), the most frequent malignant pediatric brain tumor is divided into four subgroups, i.e. wingless-type (WNT), sonic hedgehog (SHH), Group 3, and Group 4. Among them, haploinsufficiency of chromosome 17p13.3 is most commonly seen in Group 3 and 4 tumors, which are concurrently associated with the poorest prognosis. Recent studies have revealed the importance of microRNAs (miRNAs) in regulating posttranscriptional gene expression in MB tumorigenesis. In this study, we sought to identify the role of miR-212, which resides on chromosome 17p13.3, in the development of non-SHH/WNT medulloblastoma. RNA expression analysis showed significantly reduced expression of miR-212 in MB tumor cell lines and in *ex vivo* non-SHH/WNT MB tumor samples (n=16). To further elucidate its putative tumor suppressor role, miR-212 was over-expressed in non-SHH/WNT tumor cell line, HDMB03, followed by functional assays of tumor cell behavior. In these studies, significantly reduced cell proliferation, colony formation, migration and invasion were noted. Additionally, reduced levels of pAKT, a marker of cell proliferation, was confirmed in miR-212-overexpressing HDMB03 cells. Subsequent cell cycle analysis revealed increased G_0/G_1 cell cycle arrest with a concurrent reduction in the corresponding cell cycle regulatory proteins, i.e. CDK6 and cyclinD1. Myc amplification, which is facilitated by phosphorylation of serine-62 (p-c-Myc-S62), is a characteristic feature of Group 3 medulloblastoma and a marker of poor prognosis. Decreased levels of p-c-Myc-S62 (active form favoring proliferation) with a complimentary increase in p-c-Myc-T58 (inactive form favoring apoptosis) results in degradation of c-Myc protein and cellular apoptosis, a trend recapitulated in miR-212-over-expressing HDMB03 cells. In addition, the pro-apoptotic binding partners of c-Myc, i.e. Bin-1 and $P19^{ARF}$, were noted to be upregulated in miR-212 over-expressed HDMB03 cells, further favoring apoptosis. That c-myc may also serve as a target of miR-212 is currently being studied. These results substantiate miR-212 as a tumor suppressor gene in non-SHH/WNT medulloblastoma.

PDTM-09. DIFFUSE INTRINSIC PONTINE GLIOMA AND PEDIATRIC GLIOBLASTOMA DERIVED-EXOSOMES HAVE SPECIFIC ONCOGENIC SIGNATURES

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Diffuse intrinsic pontine glioma (DIPG) and pediatric glioblastoma (pGBM) are heterogeneous brain tumors characterized by different anatomical and molecular subgroups and the presence of genetically and phenotypically distinct subclonal cell populations. It is recognized that exosomes mediate cross-talk among tumor cells. We hypothesize that there are different exosome-mediated paracrine signaling promoting tumour progression in DIPG and pGBM. Our aim was to determine the specific DIPG and pGBM-derived exosome oncogenic signatures. We used a panel of fifteen patient primary-derived cell lines, which included nine DIPG (seven H3.3 K27M, one H3.3 K27M/ACVR1 and one H3.1 K27M/ACVR1), one diffuse midline glioma H3.3 K27M and three GBM (one H3.3 G34R and two histone WT). Conditioned medium was collected from cells maintained under stem-cell culture condition, adherent on laminin and/or as neurospheres (NS), and exosomes harvested through serial centrifugations. Electron microscopy demonstrated that the isolated microvescicles are exosomes sized between 50–80 nm. DIPG derived-exosomes appeared to have a variable cargo of total protein (μg)/10^6 cells, which was higher than for pGBM-exosomes. Proteomic analysis revealed that proteins associated with vesicle docking, exocytosis and synaptic transmission were exclusively enriched in pontine-derived exosomes, while cell-cell and cell-matrix interaction proteinswere exclusive tohemispheric ones. Proteins in common to the two locations were involved in metabolism and energy pathways. Interestingly, principle component analysis on the different molecular subgroups suggests that ACVR1 may be not implicated in the exosomal proteomic signature. Exosomal miRNA profile appeared to be driven by the two main histone mutated subgroups H3.3 K27M and H3.1 K27M with the latter overexpressing hypoxia and angiogenic-associated miRNAs, leading to distinct oncogenic programs with different specific potential therapeutic targets. This study aimed to development new diagnostic/prognostic tools for DIPG and pGBM patients. Further investigations are aimed to identify new therapeutic strategies to inhibit the cross-talk among glioma subpopulations.

PDTM-10. NOVEL RNA-TARGETING STRATEGY FOR TREATING T CELL-DRIVEN IMMUNOSUPPRESSION IN HUMAN DIFFUSE INTRINSIC PONTINE GLIOMA

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PURPOSE: Our laboratory's work in adult high-grade glioma has discovered, a therapeutically-targetable pathway through the inducible expression of indoleamine 2,3 dioxygenase 1 (IDO1). IDO1 is an immunosuppressive enzyme that metabolizes tryptophan (Trp). The premise of our project is to delineate the role of T-cell-driven IDO1 expression in human DIPG, and to develop the therapeutic potential for inhibiting human IDO1 with novel, specific, small inhibitory (si)RNA oligonucleotides. METHODS: Gene expression for human IDO1, T-cell specific, CD3e, and the proinflammatory cytokines, IFNγ and IFNb, were investigated among surgically-resected pediatric brain tumor specimens obtained from the Childhood Brain Tumor Tissue Consortium (CBTTC). IDO1 and CD3e were also explored in a novel, humanized DIPG mouse model (NSG-SGM3-BLT), by injecting SF8628 into the brain stem. Humanized mice were either untreated or depleted for human $CD4^+$ and $CD8^+$ T cells. *In vitro* analysis included unique patient-derived DIPG cell lines, stimulated with human IFNγ, and analyzed for IDO1 mRNA and protein levels, with and without the addition of IDO1 siRNA. RESULTS: IDO1 expression is normally low in cultured human DIPG, but rapidly induced by IFNγ. Analysis of surgically-resected human pediatric brain tumor specimens confirms the association between IDO1 levels and the co-expression of IFNγ and IFNb. Strikingly, human T cells directly increase human IDO1 expression in intracranial DIPG, while our siRNA specifically decreases IDO1 mRNA, protein and enzyme activity/levels. DISCUSSION: The poor prognosis of children with DIPG, combined with the lack of effective therapies, emphasizes the importance of understanding immunosuppressive IDO1 and its potential therapeutic value. These data also confirm that, immunotherapies aimed at enhancing T cell effector functions in DIPG patients, also need

to consider resulting effects on intratumoral IDO1-mediated immunosuppression. Our ongoing work aims to weaponize our novel IDO1-targeted siRNA for *in vivo* delivery, and for adjuvant treatment in DIPG patients receiving immunotherapy.

PDTM-11. A NOVEL EX VIVO MODEL FOR HUMAN MEDULLOBLASTOMA: A NEW PERSONALIZED MEDICINE TOOL

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BACKGROUND: Medulloblastoma (MB) is the most common malignant pediatric brain tumor. Current treatment involves multi-agent chemotherapy and/or radiation, but this one-size-fits-all treatment often results in long term side effects. The development of novel therapeutics has been slowed, limited by the lack of representative samples of individual patient MB; therefore, we adapted a novel drug testing assay to address this issue: organotypic *ex vivo* brain tumor slice culture (OSC). We hypothesize that OSC can be a robust real-time screening tool for evaluating drug efficacy for individual MB patients. We tested novel inhibitors for STAT3 (WP1066) and YB1 (fisetin) in an OSC assay, with cisplatin and DMSO vehicle as positive and negative controls respectively. OBJECTIVES: To determine the feasibility of performing our OSC on human MB and to evaluate WP1066 and fisetin on patient-derived samples. METHODS: MB was taken directly from the OR at time of initial resection. Tumors were sectioned into 300-µm-thick slices with a vibratome. The slices were cultured, treated with WP1066, fisetin, cisplatin, or vehicle and after 48hrs, the slices were fixed in 4% paraformaldehyde. The slices were immunostained with antibodies against cleaved caspase 3, and confocal images were obtained. Analysis and quantification of staining was done with FIJI and IMARIS imaging software. RESULTS: We were able to perform our assay on three patient samples: two non-WNT/non-SHH and one SHH tumor. WP1066 and fisetin treatment induced cell death in all three samples, similar to cisplatin, and significantly more than vehicle. We also found increased cell death in the nestin positive tumor stem-like cells. We correlated phenotypic response with gene expression, as determined by single-cell RNA-sequencing performed on one MB and whole exome sequencing of another MB. CONCLUSIONS: We have successfully performed a novel OSC assay for real-time drug testing, thus illustrating a possible new personalized medicine tool in MB.

PDTM-12. DLX2 TRANSCRIPTIONAL REGULATION OF CENTRAL NERVOUS SYSTEM CELL FATE RELEVANCE TO PEDIATRIC DIFFUSE MIDLINE GLIOMAS WITH HISTONE 3 MUTATIONS

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is refractory to current therapy with less than 2% long term survivors. The identification of histone H3.1/H3.3 K27M mutations in most DIPG has provided new insights into the biology of this fatal disease. The DLX homeobox genes are expressed in the developing forebrain. The Dlx1/Dlx2 double knockout (DKO) mouse loses tangential GABAergic interneuron migration to the neocortex. We have identified genes that encode glutamic acid decarboxylase (GAD) enzymes as direct transcriptional targets of DLX1/DLX2. In DIPG patients with H3.3 K27M mutations there is decreased *Dlx2* and increased expression of the myelin transcription factor, *Myt1*. There is loss of H3 K27 tri-methylation (me^3) expression in many tumors with K27M mutations. METHODS AND RESULTS: We used bioinformatics approaches and chromatin immunoprecipitation (ChIP) assays to identify *Olig2*, *Nkx2.2* and *Myt1* promoter sequences as candidate DLX2 targets *in vivo*. DNA binding specificity was confirmed by gel shift assays *in vitro*. The functional consequences of *Dlx2* co-expression with reporter constructs of ChIP-isolated promoter fragments of *Olig2* and *Nkx2.2* demonstrated repression of gene targets *in vitro*. qPCR showed increased *Olig2* and *Nkx2.2* expression in the DKO forebrain. Stable transfection of *Dlx2* into a murine DIPG (mDIPG) cell line with the K27M mutation resulted in increased expression of *Gad1* and *Gad2* and decreased expression of *Olig2* and *Nkx2.2*. *Dlx2* stable transfection also resulted in decreased migration, invasion and colony number and size *in vitro*. Of significance, we demonstrated decreased expression of H3.3 K27M and restoration of H3.3 $K27me^3$ expression in *Dlx2* stable transfected mDIPG cells. CONCLUSIONS: DLX transcription factors promote GABAergic interneuron and concomitant inhibition of oligodendroglial differentiation in neural progenitors by repression of a suite of genes including *Olig2* and *Nkx2.2*. Restoration of H3 $K27me^3$ expression in DIPG provides a promising lead towards exploration of differentiation as a therapeutic strategy for DIPG.

PDTM-13. OVEREXPRESSION OF MYC ALONE IS SUFFICIENT TO INITIATE GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant brain tumor in children. Among the four molecular subgroups of MB (WNT, SHH, Group 3 and Group 4), patients with Group 3 MB exhibit the worst prognosis. Group 3 MB is associated with amplification and overexpression of the *MYC* oncogene. However, whether *MYC* overexpression alone is sufficient to induce Group 3 MB tumorigenesis in specific cell type(s) in the cerebellum remains unclear. The study of the etiology of Group 3 MB and the development of effective, targeted therapies for this disease has also been impeded by lack of appropriate disease models that faithfully recapitulate Group 3 MB. Here, we generated a novel mouse model for Group 3 MB and demonstrated that overexpression of *MYC* alone is sufficient to transform astrocyte progenitors and granule neuron progenitors in the early postnatal cerebellum. The resulting tumors resemble human Group 3 MB in terms of histology and gene expression profiles, making this animal model a valuable tool for the development and testing of new therapies. To identify potential therapeutic targets, we analyzed dysregulated gene expression and revealed that the genes involved in the glucose metabolism pathways were significantly upregulated in murine and human Group 3 MB compared to normal cerebellar cells or SHH Group MB. Among these genes, expression of lactate dehydrogenase A (LDHA), which catalyzes the conversion of pyruvate to lactate during energy metabolism, is associated with poor prognosis in Group 3 MB. Inhibition of LDHA by either RNA interference or pharmacological agents significantly reduced growth of both mouse and human Group 3 tumor cells, without affecting SHH Group MB, suggesting that LDHA is a potential specific target for treating Group 3 MB.

PDTM-14. MiR-1253 IS A NOVEL TUMOR SUPPRESSOR GENE IN MEDULLOBLASTOMA

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Medulloblastoma (MB), the most common malignant pediatric brain tumor, is a leading cause of childhood related morbidity and mortality. Large-scale transcriptional profiling and mutational analyses have facilitated the stratification of medulloblastoma into four primary subgroups, i.e. SHH (sonic hedgehog), WNT (wingless), and non-SHH/WNT groups 3 and 4. The most frequent cytogenetic abnormality in medulloblastoma, i.e. i17q, distinguishes the non-SHH/WNT subgroup. Haploinsufficiency of 17p13.3 is reported in up to 50% of human MB cases. Included within this locus is miR-1253, which is exclusively expressed in the brain and an important regulator of bone morphogenic proteins that play a critical role in cerebellar development. Recently, two oncogenic targets of miR-1253, i.e. TGIF2 and ALX4, were identified in SHH medulloblastoma. Based upon these observations, we hypothesized that miR-1253 may be a putative tumor suppressor gene that undergoes epigenetic silencing in pediatric medulloblastoma. We first discovered reduced expression of miR-1253 in 24 pediatric medulloblastoma specimens and in 7 medulloblastoma cell lines. We then learned that miR-1253 silencing is accomplished via hypermethylation; expectedly, de-methylation of miR-1253, resulted in the recovery of expression with a subsequent decline in MB cell proliferation. MiR-1253 restoration was further concomitant with activation of apoptotic pathways and cell cycle arrest at G_0/G_1 phase. Moreover, miR-1253 overexpression led to a reduction in cell proliferation, colony formation, migration and invasive potential of MB tumor cell lines. Using high throughput RNA sequencing analysis and luciferase reporter assay, we further identified several oncogenic targets of miR-1253, including CDK-6 and CD276. Taken together, these data strongly support a tumor suppressive role for miR-1253. This would be the first time such an effect has been attributed to miR-1253 in the context of medulloblastoma.

PDTM-15. IDENTIFICATION AND CHARACTERIZATION OF WILMS' TUMOR PROTEIN IN PEDIATRIC MIDLINE GLIOMAS

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Pediatric midline gliomas, especially those associated with the pons, known as diffuse intrinsic pontine gliomas (DIPGs) are deadly pediatric brain cancer that makes up 10–15% of all central nervous system (CNS) tumors in children. Its anatomical location and infiltrative nature makes it one of the most challenging tumors to treat. Targeted therapies are gaining more interest in CNS tumors. Identification of tumor associated antigens is one of many requirements in developing an effective targeted therapy. Wilms' tumor protein (WT1) has been ranked number 1 cancer immunotherapy target by National Cancer Institute. Many types of solid tumors have been shown to express WT1 and it is being examined as one of potential immunotherapeutic targets. Here we validated WT1 as a potential tumor associated antigen in pediatric diffuse midline gliomas using formalin fixed paraffin embedded (FFPE) tumor and adjacent healthy specimens, fresh frozen post-mortem tissues and patient-derived DIPG primary cell lines. Our immunohistochemistry (IHC) staining of patient FFPE specimens showed strong WT1 immunoreactivity in tumor compared to adjacent normal tissue. Western blot of tumor tissues and cell lines were performed to further validate WT1 levels in the tumors versus adjacent healthy tissues. Interestingly, tumors showed cytoplasmic expression of WT1. In addition, H3.1K27M subtype gliomas showed weak to absent WT1 immunoreactivity compared to strong to moderate in H3.3K27M subtypes. Western blots also validated the differential expression of the protein. Our study suggests that WT1 is a potential tumor specific antigen in pediatric midline gliomas, which can be utilized for targeted therapies such as immunotherapy.

PDTM-16. AN IMPROVED DIFFUSE INTRINSIC PONTINE GLIOMA MODEL INITIATED IN OLIG2-EXPRESSING PROGENITORS OF THE NEONATAL BRAINSTEM

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Diffuse intrinsic pontine glioma (DIPG) is an incurable brain tumor that arises in the pons of children. Recent studies using single cell RNAseq and enhancer analysis of DIPG tumor cells, together with analysis of the developing human pons, strongly suggest that an oligodendrocyte progenitor cell is the most likely cell-of-origin for DIPG. As there is a need for immunocompetent DIPG models that arise in the correct cell-of-origin (spatiotemporally) and recapitulates the genetic alterations of the human disease, we sought to develop a DIPG mouse model that arises in Olig2-expressing progenitors of the neonatal brainstem. Here we describe a novel mouse model by expressing PDGF-B, with H3.3K27M or H3.3 wild-type in Olig2-expressing progenitors of the neonatal brainstem using Olig2-tva-cre;p53fl/fl mice. Although both H3.3K27M tumors and H3.3 wild-type tumors have high rate of Ki-67 and Olig2 positivity, H3.3K27M tumors show a higher rate (100%) of leptomeningeal dissemination than H3.3 wild-type tumors (50%) and mice harboring H3.3K27M tumors demonstrate significantly shorter survival periods than those harboring H3.3 wild-type tumors (28 days vs. 37 days, p=0.048). Interestingly, expression of PDGF-B and H3.3K27M in Olig2-expressing progenitors of the neonatal brainstem of Olig2-tva-cre;p53fl/+ demonstrate dramatically delayed gliomagenesis relative to Olig2-tva-cre;p53fl/fl mice (28 days vs. 119 days, p<0.0001) illustrating that p53 status (heterozygous vs. homozygous deletion) impacts survival more dramatically than H3.3K27M. Ongoing studies will compare the transcriptome of H3.3K27M mutant tumors to H3.3 wild-type tumors and to human tumors harboring H3.3K27M, PDGFRA amplification and p53 mutations to further credential this model.

PDTM-17. DUAL TARGETING OF SHH SIGNALING AND BCL-xL FUNCTION AS A NOVEL TREATMENT FOR MEDULLOBLASTOMA

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Medulloblastoma is markedly more sensitive to radiation and chemotherapy than other malignant brain tumors. We have previously shown that medulloblastomas radiation sensitivity of depends on the function of the internal apoptosis pathway. We tested whether this pathway could be directly activated, without radiation, by targeting the anti-apoptotic protein BCL-xL. Conditional deletion of *Bcl-xL* in in cerebellar progenitors induced precipitous apoptosis as these cells exited the cell cycle. *Bcl-xL* deletion in SHH-driven medulloblastomas in SmoM2 mice also impaired tumor growth and caused focal regions of tumor necrosis. However, *Bcl-xL*-deleted medulloblastomas eventually progressed. Expression microarray analysis showed increased Cdk2 in *Bcl-xL*-deleted tumors, suggesting that *Bcl-xL* deletion selected for tumor cells that rapidly re-entered the cell cycle. Consistent with the idea that proliferating cells are relatively protected from BCL-xL dependence, we found that proliferating cerebellar progenitors evade apoptosis through SHH-dependent expression of the BCL-xL homolog MCL-1. To address this potential mechanism of resistance in on-going work, we are combining *Bcl-xL* deletion with SHH inhibition, testing the hypothesis that forced cell cycle exit will reduce MCL-1 and sensitize medulloblastomas to BCL-xL disruption. Based on our data, we propose combined targeting of the SHH signaling and BCL-xL function as a new approach to medulloblastoma treatment.

PDTM-18. ACTIVATION OF TUMOR-REACTIVE T CELLS AGAINST BRAIN STEM GLIOMA USING HEMATOPOIETIC STEM CELLS

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INTRODUCTION: Adoptive cellular therapy is demonstrably efficacious in two preclinical models of brain stem glioma (BSG), OB1 (wildtype H3.3), and K2 (H3.3K27M mutation). The survival benefit of adoptive cellular therapy is significantly enhanced by concomitant transfer of bone marrow derived hematopoietic stem cells (HSC) with tumor-reactive T cells. We demonstrate in orthotopic models that HSC-derived cells differentiate into dendritic cells within the tumor microenvironment and cross-prime adoptively transferred T lymphocytes in BSG. Using a novel method of 3D printing to fabricate microtumors using OB1 and K2 cells, we interrogate the BSG tumor microenvironment and demonstrate direct activation of tumor-reactive T cells *in situ* by HSC-derived dendritic cells. METHODS: We employ orthotopic K2 and OB1 BSGs as well as an *in vitro* system using an innovative technique where 3Dtumoroids are fabricated using K2 and OB1 cells into engineered medium that allows in situ imaging over >30 days. Tumor-reactive T cells and HSCs are also applied to the system. T cells were generated using mice with fluorescent reporters. High resolution imaging is used for visualization of cell-to-cell interactions. Flowcytometry and immunofluorescence is used to confirm T cell and HSC immunophenotypes and activation status. RESULTS & CONCLUSION: In orthotopic models of K2 and OB1, we found that HSCs are required for T cell infiltration to BSGs. T cell infiltration into BSG is an impactful observation in this tumor type. 3D models of BSG, tumor-reactive T cell infiltration was significantly increased in the presence of HSCs. Using T cells generated from GREAT mice which have YFP reporter on the IFNγ promoter, we visualized direct activation of tumor-reactive T cells *in situ*. These studies are unique in that the *in situ* interrogation of BSG is not possible due to location. Here were are able to bypass this limitation with this technology to make key immunological observations.

PDTM-19. TUMOUR TREATING FIELDS (TTFIELDS) EXHIBIT EFFICACY ON HIGH-GRADE PAEDIATRIC BRAIN TUMOUR CELL LINES

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INTRODUCTION The EF-14 trial of the Optune system which utilises Tumour Treating Fields (TTFields) has shown positive results in adult Glioblastoma multiforme (GBM) patients. These results have given strength to the feasibility of TTFields being added to existing chemotherapy and radiation therapy for brain tumour patients. Here we present the data showing the efficacy of TTFields on a panel of paediatric GBM, Medulloblastoma and Ependymoma cell lines. METHODS The Inovitro system is the laboratory testing system used to develop the clinically approved Optune system. Inovitro was used to deliver TTFields over a range of clinically relevant frequencies (100-400kHz) to our panel of high grade paediatric cell lines. The effects of TTFields on cell viability was assessed using metabolic viability tests, and cell cycle analysis was performed using flow cytometry. Gene expression analysis was performed via Affymetrix microarray. RESULTS TTFields have significant efficacy on all of our cell lines, and the extent of this is dependent upon frequency. Cells treated with TTFields were reseeded and growth rates were compared to control cells. The treated cells experienced up to 75% slower growth rates following treatment. Cell cycle analysis revealed that TTFields treated cells have significantly greater levels of G2/M phase accumulation relative to control, and this coincides with previous observations in adult GBM cell lines. The efficacy of TTFields may be significantly increased with the addition of the mitotic inhibitor, paclitaxel. The effects of TTFields treatment on gene expression will be discussed. CONCLUSIONS TTFields treatment has demonstrated efficacy against our panel of paediatric GBM, Medulloblastoma and Ependymoma cell lines - further investigation is warranted so that this may be translated into the clinic.

PDTM-20. ELUCIDATING MOLECULAR PATHOGENIC MECHANISMS OF THE HISTONE H3.3 G34R MUTATION IN PEDIATRIC HIGH-GRADE GLIOMAS (HGGs)

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High-grade glioma (HGG) is the most common and lethal type of primary brain tumor in humans, remaining essentially incurable; 16% of pediatric and young adult HGGs in cerebral hemispheres encode Gly34Arg/Val (G34R/V) substitutions in the histone variant H3.3. It was shown that H3.3G34R/V leads to a local downregulation of H3K36 trimethylation at specific genes' loci. Although K36me3 has been associated to various mechanisms including DNA repair, gene expression and chromatin homeostasis, the specific mechanisms by which H3.3G34R/V drive malignancy of HGGs remain largely unknown. We developed a mouse model of H3.3-G34R HGG by transfecting a transposase-expressing plasmid along with transposable cassettes to drive the expression of H3.3G34R, shRNAs against *p53* and *Atrx* (genes whose mutations co-segregate with H3.3G34R in HGGs) and NRAS, into neonatal mice. We compared H3.3G34R, NRAS, sh*p53*, sh*Atrx*, tumors (NPAH) with H3.3 wild type, sh*p53*, shATRX, tumors (NPA), and observed an increased median survival in NPAH tumors compared to NPA. To further address the role of G34R mutation in oncogenesis we integrated H3.3G34R/V into SJ-GBM2, an H3.3 wild type pediatric HGG cell culture. Wild type SJ-GBM2 cells formed tumors when intracranially injected into NSG mice, but SJ-GBM2-H3.3G34R cells did not. We observed that SJ-GBM2-H3.3G34R undergo a series of molecular changes: i.e., neural cells' dedifferentiation, evidenced by decreased levels of Olig1, Olig2 and GFAP; epigenetic reprogramming evidenced by global changes in H3K36 acetylation and H3K27 acetylation; a switch in DNA repair pathways, from homologous recombination to non-homologous end joining; and upregulation of DLX6, a transcription factor with roles in forebrain and craniofacial development. Our results indicate that H3.3G34R/V mutations may drive tumorogenesis by maintaining neural cells' undeferenciation, altering the DNA repair pathway choice and inducing epigenetic changes, with potential consequences in mutational burden and genomic stability. These characteristics could be exploited to design novel therapies.

PDTM-21. MATCHING OF SINGLE CELL TRANSCRIPTOMICS FROM CEREBELLAR DEVELOPMENT IDENTIFIES PUTATIVE SUBGROUP SPECIFIC CELLS OF ORIGIN FOR MEDULLOBLASTOMA

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We isolated cells from various points in murine embryonic and early post-natal cerebellar development (E10 to P14) and undertook single cell RNA sequencing to identify >30 transcriptionally distinct cell populations. Reconstruction of developmental lineages in the developing cerebellum through pseudotemporal analysis demonstrate that a progenitor cell population from the upper rhombic lip gives rise to both the external granule cell layer (EGL) and the previously under-studied unipolar brush cells (UBC). Unipolar brush cells are a glutamatergic interneuron most prevalent in the inferior and lateral cerebellum. Transcriptional matching of bulk human tumor RNA-seq data from human patients demonstrates subgroup specific transcriptional resemblances. As expected, Shh MBs resemble the developing EGL. Fascinatingly, only Shh tumors that transcriptionally mirror earlier, but not later EGL developmental time points were found to be metastatic in human patients. Group 3 tumors have a resemblance to early Nestin +ve stem cells across the entire subgroup, with additional similarities to the EGL, UBC, and GABAergic interneurons. Unexpectedly, Group 4 tumors were vastly most similar to UBCs, and on further analysis to a single known subset of UBCs (Calb2+ve UBCs). Single cell RNA-seq from human medulloblastomas (Shh, Group 3, and Group 4 MB) largely confirms the transcriptional similarities to murine cerebellar developmental lineages observed in the bulk RNA-seq data, but also demonstrates that most medulloblastomas have multiple distinct tumor cell clusters. Distinct single cell expression clusters from an individual MB demonstrate that there is a developmental lineage or hierarchy of cells with most tumors, that this hierarchy is likely hardwired from normal development, and that bulk tumor data in fact reflects a heterogeneous cell population. These data pinpoint possible cells of origin for medulloblastoma subgroups, illustrate a further layer of MB heterogeneity, and allow a comparison of normal and MB transcriptomes to further understand MB biology.

PDTM-22. TARGETING A NOVEL METABOLIC DEFECT IN PPM1D-MUTANT DIFFUSE INTRINSIC PONTINE GLIOMAS

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Somatic truncating mutations within the oncogenic phosphatase PPM1D, have been identified in diffuse intrinsic pontine glioma (DIPG) and other high grade pediatric brain tumors. These stabilizing mutations result in an overabundance of the mutant protein within tumor cells, and inactivation of important DNA damage response (DDR) and cell cycle checkpoint targets. Despite our current understanding of these mutations, there exists no viable therapeutically viable strategy for the treatment of PPM1D-mutant tumors. Therefore, we sought to further explore the oncogenic potential of these truncating mutations, and to identify selective inhibitors of mutant DIPGs. Using isogenic astrocyte pairs with containing engineered-PPM1D mutations, we characterized the phenotypic effects of these genetic alterations on DDR and DNA repair processes. We identified numerous DNA repair defects that likely can be exploited for a therapeutic gain, including accelerated gH2AX dephosphorylation, which correlated with despite an intact 53BP1 DNA damage response (DDR), which correlated with intrinsic radiosensitivity. We then performed a small molecule screen with inhibitors of DDR-related proteins to identify novel synthetic lethal pathways in PPM1D-mutant DIPGs. These studies revealed an unexpected, clinically actionable target in PPM1D-mutant cells, involved in global cellular metabolism. This induced sensitivity was detected using multiple drugs targeting the same pathway, and was exquisitely selective, with a 10^4 –fold difference in sensitivity between mutant and wild type cells. Further, upon testing these compounds against patient-derived, PPM1D-mutant DIPG neurospheres, we again found remarkable levels of sensitivity, demonstrating their effects in relevant preclinical models. We will present the mechanistic basis for this described synthetic lethality, which implicates key epigenetic alterations and gene expression changes, ultimately resulting in the described metabolic defect. Overall, our novel discovery provides exciting new insights into the biology of PPM1D mutations and the treatment of these devastating pediatric diseases.

PDTM-23. CD57 DEFINES A NOVEL MARKER OF GLIOBLASTOMA STEM CELLS THAT DRIVES THE INVASION OF GBM

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Glioblastoma multiforme (GBM) is the most lethal brain tumor. Diffuse invasion is one of the important biologic features that make GBM particularly difficult to treat. While existing studies on GBM invasion are primarily conducted in tumor core tissues from surgical resections, it is unclear whether infiltrative GBM cells would be more informative for studying their invasive nature, and little is known if and which cancer stem cell populations are driving GBM invasion. To address these issues, we utilized 6 patient tumor-derived orthotopic xenograft mouse models to isolate invasive GBM cells (GBM^{INV}, infiltrating normal mouse brain parenchyma) and tumor core GBM cells (GBM^{TC}) and compared their biological features. Our result showed that the GBM^{INV} cells have stronger neurosphere forming efficiency *in vitro*, more tumorigenic capacity *in vivo*, even some invasion-related genes also be detected to be changed in GBM^{INV} cells. In the further study, gene profiling showed that CD57 is higher expressed in mostly GBM models and in patient tissues; CD57+ cells have stronger neurosphere formation ability with compared to CD57- cells; The survival time of mice injected with CD57+ cells decreases compared with mice with CD57- cells. Furthermore, CD57+ cells in GBM^{INV} is higher expressed in PDOX invasive tumor cells than the expression of GBM^{TC} cells; CD57+ cells in GBM^{INV} cells are enriched (>2 folds) and have stronger neurosphere formation ability and more invasive with compared to GBM^{TC} cells in mostly GBM models; The survival time of mice injected with GBM^{INV} cells decreases compared with mice with GBM^{TC} cells. We found that CD57+ cells expressed high levels of self-renewal genes (BMI1 and Nanog). In conclusion, we showed that invasive GBM cells were not biologically identical to the matched tumor core cells and identified CD57 as a novel stem cell marker that was associated with GBM infiltration.

PDTM-24. PILOT STUDY OF CIRCULATING TUMOR CELLS IN PEDIATRIC HIGH GRADE BRAIN TUMORS

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BACKGROUND: Brain tumors are the second most common malignancy in children, and are the leading cause of death from childhood cancer. Although significant strides have been achieved in improving survival of most childhood malignancies, the outcome for children with brain tumors has remained poor. Advanced imaging techniques can reduce diagnostic uncertainty, but the need to identify better disease markers is immense and remains unmet. In that regard, analysis of circulating biomarkers is a novel and promising avenue for monitoring disease status. Despite increasing use in tumor research, circulating tumor cells (CTCs) have not been studied in pediatric brain tumor and only scarcely investigated in adult brain tumors. METHOD: Based on previous research showing cell surface vimentin (CSV) as a key surface marker for CTC detection, we developed an automated CSV-based CTC capture method for pediatric brain tumor using the Abnova CytoQuest CR CTC isolation platform. With this platform, we processed PBMCs isolated from whole blood samples through the automated system to trap CSV-positive CTCs on to a chip. Captured cells are then stained for CSV and CD45 before automated scanning and detection to determine CTC yield per sample. RESULT: Ten patients were consented on the study, nine patients (3 high grade glioma, 3 brain stem glioma, 1 pineoblastoma, 1 medulloblastoma, 1 atypical teratoid rhabdoid tumor) had an adequate samples for testing, of those 7 had CTC detected from PBMCs. Our results show that brain tumors patients can and do exhibit CSV+ CTCs. These findings do not distinguish between various types of brain malignancies as evidenced by positive CTC isolation in multiple types of gliomas and embryonal tumors. CONCLUSION: Overall, we present the first study of CTCs in pediatric tumors using an automated approach. This is a promising methodology for future tumor risk stratification and treatment response monitoring.

PDTM-26. DUAL THERAPY WITH PI3K INHIBITOR ZSTK-474 AND MEK INHIBITOR TRAMETINIB VIA CONVECTION-ENHANCED DELIVERY IN A GENETICALLY-ENGINEERED MOUSE MODEL OF DIFFUSE INTRINSIC PONTINE GLIOMA

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is a pediatric brain tumor with a median survival of less than one year. Prior studies have highlighted PI3K/AKT signaling amplifications in DIPG. However, compensatory activation of parallel pathways, e.g. MAPK, may lead to resistance to PI3K inhibition. We evaluated whether dual therapy with PI3K and MEK inhibitors would synergistically inhibit DIPG growth *in vitro* and prolong survival in a genetically-engineered mouse model. METHODS: Three patient-derived (SU-DIPG-IV, SU-DIPG-XIII, and SF8628) and one mouse-derived cell line were treated with ZSTK-474 and trametinib. Synergy was analyzed using Chou-Talalay combination index (CI). *In vivo* experiments introduced H3.3K27M mutations, PDGF-B overexpression, and p53 loss via RCAS-tva in Nestin-tva mice. Animals were treated with local convection-enhanced delivery (CED) of ZSTK-474 and trametinib at IC90 concentrations (both 1.9uM). RESULTS: ZSTK-474 and trametinib reduced cell proliferation across all cell lines (IC50s 0.75uM, 0.77uM, 0.37uM, and 0.12uM for ZSTK-474; 0.09uM, 0.01uM, 0.52uM, and 0.27uM for trametinib). Combination treatments were found to be synergistic (CI<1) across cell lines at tested concentrations, with the exception of SU-DIPG-XIII above 1uM. Western blot analysis of SU-DIPG-XIII demonstrated decreased AKT phosphorylation and increased ERK phosphorylation (downstream targets of PI3K and MEK) when treated with ZSTK-474, increased pAKT and decreased pERK with trametinib, and decreased pAKT and pERK with combined treatment. *In vivo* experiments revealed a trend towards prolonged survival versus vehicle (median survival 41 versus 31.5 days post-induction, Gehan-Breslow-Wilcoxon $\chi 2 = 2.7$, $p = 0.10$). Importantly, however, no symptoms of neurotoxicity from CED were observed. CONCLUSION: Dual therapy with ZSTK-474 and trametinib synergistically inhibited DIPG growth *in vitro*. Preliminary *in vivo* results demonstrated a trend towards prolonged survival. Novel local CED of ZSTK-474 and trametinib resulted in no short- or long-term neurotoxicity at current dosing, indicating increased dosage or treatment frequency may prolong survival in future work.

PDTM-27. EPIGENETIC LOSS OF BAI1 EXPRESSION IN CEREBELLAR GRANULE NEURON PRECURSORS INACTIVATES THE p53 TUMOR SUPPRESSOR AND FACILITATES MEDULLOBLASTOMA FORMATION IN THE CEREBELLUM

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Adhesion G-protein coupled receptors (ADGRs) are transmembrane proteins involved in cell-cell/matrix interactions. We show *ADGRB1* gene, which encodes Brain-specific angiogenesis inhibitor 1 (BAI1), is epigenetically silenced in human medulloblastomas through a methyl-CpG binding protein 2 (MBD2)-dependent mechanism. Knockout of *Adgrb1* in mice augments proliferation of cerebellar granule neuron precursors (GNPs), and increases medulloblastoma penetrance and accelerated death in *Ptch1*$^{+/-}$ mice. BAI1 prevents Mdm2-mediated p53 polyubiquitination, and its loss substantially reduces p53 levels. Reactivation of BAI1/p53 signaling axis by targeting MBD2 pathway suppresses human medulloblastoma growth in orthotopic xenograft models. Our findings highlight the importance of BAI1 loss in medulloblastoma and demonstrate that epigenetic restoration of its expression with a new brain-permeable MBD2 inhibitor has therapeutic potential. Revealing BAI1 as a physiological tumor suppressor in medulloblastoma unveils a direct crosstalk between ADGRs and p53 signaling, and provides a causal relationship between ADGRs and cancer. The discovery of a novel upstream regulator of the p53 tumor suppressor is highly significant because of this pathway's involvement in many cancers. Disruption of the BAI1/mdm2/p53 signaling axis through BAI1 silencing reveals a vulnerability in cancer, and offers an opportunity for therapeutic exploitation through epigenetic reactivation. We provide proof-of-principle that this can be achieved with a chemical scaffold targeting MBD2, and this lead molecule is actionable for translation into a first-in-class therapeutic intervention against medulloblastoma, and possibly other cancers (Zhu D *et al*, Cancer Cell, in press).

PDTM-28. THE CONTRIBUTION OF PAX GENES AS NOVEL TUMOR SUPPRESSORS IN GROUP 3 MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant primary pediatric brain tumor. Of the four distinct molecular MB subgroups, Group 3 MB has the worst prognosis, is highly metastatic, and is poorly defined with regard to the pathways that contribute to tumor progression. OTX2 is overexpressed/amplified in 80% of Group 3 MB, where it contributes to increased self-renewal and reduced neuronal differentiation. In order to identify genes associated with an OTX2 gene regulatory network in Group 3 MB cells, we mapped changes in active (H3K4me3) and repressive (H3K27me3) histone modifications following OTX2 silencing in Group 3 MB tumorspheres by ChIP-sequencing. Genes with significant changes in H3K27me3 profiles were associated with neuronal differentiation while genes that had significant changes in H3K4me3 profiles were associated with cell cycle progression. Interestingly, significant loss of H3K27me3 was observed for 114 transcription factors, indicating an overall de-repression of transcription factor expression following OTX2 silencing. Members of the PAX gene family were among the transcription factors de-repressed upon OTX2 silencing, and their role in MB progression has not been explored. Expression analysis in a cohort of 763 patient samples demonstrated that *PAX3* and *PAX6* expression is significantly lower in Group 3 MB. Reduced expression of *PAX3* and *PAX6* in Group 3 MB correlated with a significant reduction in overall patient survival. Following OTX2 knockdown in established and newly derived Group 3 MB cell lines, significant increases in *PAX3* and *PAX6* expression were observed providing further support for our ChIP-sequencing data. Finally, silencing of PAX6 expression resulted in an increase in cell growth suggesting a putative tumor suppressor role in Group 3 MB. Our ongoing and future studies include the further evaluation of putative divergent regulatory roles of OTX2 and PAX6 controlling neuronal differentiation in Group 3 MB.

PDTM-29. CSF H3F3A K27M CIRCULATING TUMOR DNA COPY NUMBER QUANTIFIES TUMOR GROWTH AND TREATMENT RESPONSE

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Primary brain tumors and CNS metastases shed circulating tumor DNA (ctDNA) into the CSF, which can be assessed for tumor-associated mutations. Thus far, there have been no extensive studies using droplet digital PCR (ddPCR) to detect and quantify ctDNA in the CSF of pediatric high-grade brain tumor patients. There are also gaps in our knowledge, including the potential dependence of ctDNA amount on location of sample collection and whether ctDNA can be used to quantify tumor growth and treatment response. To address these questions, we developed a novel *H3F3A* K27M ddPCR assay and applied it to four pediatric patients with *H3F3A* K27M-mutant DIPG and GBM. We found that ddPCR was able to detect the K27M mutation in patient CSF and that the closest relation emerged between mutant K27M copies per ng of total DNA (henceforth K27M copies) and contrast-enhancing tumor area on MRI. Multi-focal CSF sampling at autopsy of a DIPG patient exhibited differences in K27M copies by proximity to the tumor. To better understand changes in K27M copies in response to both growth and treatment of DIPG, we developed an *in vitro* system comprised of astrocytes (NHAs) co-cultured with luciferase-expressing human DIPG cell line DIPG007 as a means to simulate ctDNA release into the CSF. We found that DIPG007 cells released ctDNA into culture media in proportion to their proliferation, even when the media was changed frequently to approximate the constant production and resorption of CSF. Irradiation with 8 Gy resulted in a spike in mutant ctDNA 72–120 hours post-radiotherapy before decreasing. In summary, our study suggests that *H3F3A* K27M copies in the CSF of children with high-grade brain tumors have a linear relation with contrast-enhancing tumor area and that ddPCR can be used to follow treatment response including ctDNA release shortly after effective therapies.

PDTM-31. DRUG SCREENING LINKED TO MOLECULAR PROFILING IDENTIFIES NOVEL DEPENDENCIES IN PATIENT-DERIVED PRIMARY CULTURES OF PAEDIATRIC HIGH GRADE GLIOMA AND DIPG

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Paediatric high grade glioma and diffuse midline glioma (including DIPG) are comprised of multiple biological and clinical subgroups, the majority of which urgently require novel therapies. Patient-derived *in vitro* primary cell cultures represent potentially useful tools for mechanistic and pre-clinical investigation based upon their retention of key features of tumour subgroups under experimental conditions amenable to high-throughput approaches. We established 21 novel primary cultures derived from patients in London, Dublin and Rome, and together with cultures shared from Barcelona, Brisbane and Stanford we assembled a panel of 42 models under 2D (laminin matrix) and/or 3D (neurospheres) conditions, fully credentialed by phenotypic and molecular comparison to the original tumour sample (methylation BeadArray, panel/exome sequencing, RNAseq). Screening against a panel of ~400 approved chemotherapeutics and small molecules, we identified specific dependencies associated with tumour subgroups and/or specific molecular markers. This allowed for functional annotation of distinct variants in human tumours, for example cells with sensitizing (HSJD-GBM-001, *PDGFRA*_A385ins; HSJD-DIPG-008, *PDGFRA*_D846N) or resistance (HSJD-GBM-002, *PDGFRA*_D842Y) mutations to a range of PDGFRA inhibitors. We found individual models showing profound sensitivity to distinct kinase inhibitors based upon cell-specific mechanisms of activation, such as QCTB-R006 to multiple FGFR-targeted drugs, and HSJD-DIPG-012 to those directed against EGFR. Subclasses with functionally relevant pathway-based dependencies included sensitivity of DIPGs with *PPM1D*mutation (HSJD-DIPG-008, HSJD-DIPG-014) to PARP and MDM2 inhibitors, and MAPK-dysregulated PXA-like cultures (ICR-CXJ-008, ICR-CXJ015) differentially responsive to inhibitors of upstream signalling *via*PKC and CK2. Of note, all cultures were insensitive to temozolomide. In total, 85% cells were found to have at least one drug screening hit in short term assays linked to the underlying biology of the patient's tumour, providing a rational approach for individualised clinical translation.

PDTM-32. THE NOVEL THERAPEUTIC CURCUMIN ENHANCES TARGETED BACTERIOPHAGE MEDIATED IN-VITRO CELL DEATH IN PRIMARY HUMAN DIFFUSE INTRINSIC PONTINE GLIOMA

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INTRODUCTION: Diffuse intrinsic pontine glioma (DIPG) is one of the most aggressive paediatric brainstem tumours, with a dismal prognosis and a 90% mortality rate within 2 years. Over the last 50 years, DIPG patients have seen no increase in survival, owing to progress being hindered by a lack of tissue available for *in-vitro* study, the critical anatomical location of the pons and the impermeability of the blood-brain barrier (BBB). In this *in-vitro* study, we describe the use of cancer gene therapy using the targeted-bacteriophage vector to deliver the therapeutic *TNF*α transgene to DIPG cells, in combination with the novel therapeutic, curcumin, which constitutes the spice turmeric (*Curcuma longa*). MATERIALS AND METHODS: We evaluated the *in-vitro* cytotoxicity of curcumin in 2D tissue cultures of primary DIPG cells, with cell-viability measured after treatment with varying concentrations of curcumin for 72 hours. Next, we assessed the gene transduction and cell-killing efficacy of the targeted-hybrid bacteriophage, using the *Luciferase* and *TNF*α transgenes, at day 3 post-transduction. Lastly, we assessed the effect of combination therapy by adding concentrations of curcumin to DIPG cells at day 3 post-transduction by the bacteriophage, once again evaluating *Luciferase* expression and cell-killing. DISCUSSION: Here, we report for the first time, the *in-vitro* cytotoxicity of curcumin in this DIPG cell line, with significant cell death seen at as low as 37.5μM. Secondly, we show that primary DIPG cells can be successfully transduced with the targeted-modified bacteriophage vector, containing the TNFα gene, leading to TNFα-mediated cell death. Lastly, we show that the combination of curcumin with the targeted-bacteriophage vector led to enhanced DIPG cell death, due to increased bacteriophage-mediated transgene expression. CONCLUSION: We have shown that combination chemovirotherapy of curcumin and the targeted bacteriophage is efficacious *in-vitro*, warranting further *in-vivo* work to assess the safety and efficacy after systemic delivery.

PDTM-33. ATRX LOSS CONFERS ENHANCED SENSITIVITY TO COMBINED PARP INHIBITION AND RADIOTHERAPY IN PAEDIATRIC GLIOBLASTOMA MODELS

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Paediatric high grade glioma (pHGG) are defined by recurrent mutations in H3 histones, as well as frequent alterations in the SWI/SNF chromatin remodelling gene *ATRX* (α-thalassemia mental retardation X-linked), although the precise role of ATRX in tumorigenesis remains unclear. We sought to explore this using genomic analysis of patient samples, CRISPR-Cas9 engineered isogenic ATRX knockout (KO) cell lines and primary-patient-derived cultures. In combined retrospective and prospective cohorts of pHGG samples, we found *ATRX* mutations in 95/510 (18.6%) cases (27% hemispheric glioblastoma, 13% diffuse midline glioma), with the majority of truncating mutations found in the ADD domain, and missense mutations almost exclusively in the helicase domain. *ATRX* mutations commonly co-segregate with H3.3G34 and *TP53* mutations, and define a subgroup of patients with a longer overall survival, though with a greater number of somatic mutations and copy number alterations than wild-type cases. CRISPR/Cas9-mediated ATRX KO targeting the ADD domain in *TP53* mutant paediatric glioblastoma cells lead to loss of imprinting at the *H19* locus in concert with upregulation of a pro-invasive transcriptional programme, though a slower rate of orthotopic tumour growth *in vivo*. ATRX deficient cells showed an abrogated DNA damage response, with prolonged accumulation of gH2AX foci after irradiation, and an increased dependency on PARP1 through persistent parylation and stalled replication forks. Screening ATRX-deficient isogenics and patient-derived cells against a library of >400 chemotherapeutics and small molecules identified a specific dependency for ATRX loss and sensitivity to distinct PARP inhibitor chemotypes, including catalytic inhibitors (olaparib, rucaparib), and PARP trappers (talazoparib). ATRX deficiency further conferred an enhanced radiosensitization of olaparib *in vitro* and a prolonged survival of mice treated with combined PARP inhibition and radiotherapy *in vivo*. These data suggest a synthetic lethality

for PARP inhibitors in ATRX-deficient pHGG, and may represent a novel therapeutic strategy for these highly aggressive tumours.

PDTM-34. TARGETING H3.3G34R/V RE-WIRING OF THE EPIGENOME IN PAEDIATRIC GLIOBLASTOMA OF CHILDREN AND YOUNG ADULTS

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Around 15% of cerebral hemispheric glioblastomas (GBM) of children and young adults harbour G34R/V mutations in *H3F3A*, encoding the histone H3.3 variant. These tumours have a peak incidence in late adolescence but are found up to 30yrs of age, and have conflicting data as to their prognosis compared to histone wild-type cases. As little is known about its role in tumorigenesis, we sought to explore the underlying biology of H3.3G34R/V mutant GBM and identify novel avenues for therapeutic intervention through genomic, epigenomic and drug screening approaches in primary-patient-derived cultures. In combined retrospective and prospective cohorts of pHGG samples, H3.3G34R/V tumours formed a highly distinct methylation subgroup marked by global hypomethylation. They are enriched in several whole chromosomal arm DNA copy number losses, which at 4q appears to converge along with somatic mutation on the F-box protein family member *FBXW7*, known to play a role in MYCN stabilization. *MYCN* was previously identified to be differentially bound by H3K36me3, and we further identified a novel *MYCN* signature to be highly expressed in H3.3G34R/V tumours. Expression of the H3.3G34R/V mutation in pGBM cells leads to loss of H3K36me3 in *cis*, and differential genomic binding of the activating mark globally. This appears linked to distinct H3K36me3 enhancer profiles by ChIP-seq, with consistent targeting of lysine 36 demethylases *KDM2A*/*KDM4A*. Additional super-enhancers identified in H3G34R/V cells include *NOTCH1* and *SF3A2*, with both Notch signalling and splicing factor gene expression signatures significantly upregulated in patient samples. Screening against a library of >400 chemotherapeutics and small molecules identified a specific dependency for H3.3G34R/V cells on several agents targeting AURKA (as previously linked to MYCN upregulation) but also multiple chemotypes of proteasome inhibitors and the survivin inhibitor YM155. These data identify new rationally-based therapeutic options for exploration in this subset of paediatric/young adult GBM.

PDTM-35. GENETIC ALTERATIONS DRIVING SPINAL INTRAMEDULLARY METASTASES OF A HISTONE-MUTATED DIFFUSE MIDLINE PINEAL GLIOMA

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Diffuse midline gliomas are pediatric high-grade astrocytic gliomas with poor prognosis. The majority arise from the pons but may originate from other midline structures such as the hypothalamus, thalamus, and pineal region. While these tumors may spread along fiber tracts to local sites, metastasis is extremely rare and the genetic alterations underlying distant spread are poorly understood. A thirteen-year old male presented to us with worsening headaches, nausea, and vomiting and was found to have a pineal mass, for which biopsy showed diffuse midline glioma, WHO grade IV, harboring the H3K27M mutation. He subsequently underwent a suboccipital craniotomy for maximal resection, followed by proton beam radiation and concomitant temozolomide. One year later, he developed worsening lower extremity weakness for which MRI demonstrated bulky intramedullary tumor spread throughout his spine. He subsequently underwent tumor debulking at the cervical and lumbar levels. Pathology confirmed distant metastatic spread. Whole exome sequencing was performed for his initial pineal and recurrent spinal specimens, which demonstrated preservation of key driver mutations including FGFR1 activation mutation, loss of NF1, and the H3K27M mutation. However, there were additional gained copy number variation/loss of heterozygosity (LOH) events during recurrence including LOH of chr1q, which overlapped with the H3F3A locus resulting in increased variable allele frequency for the mutant allele and subsequent greater abundance of mutated H3F3A. Likewise, there was amplification of chr2p, an event overlapping with the oncogene MYCN. Finally, there was an increased mutation burden with increased C>T transition ratio although there was no evidence of mismatch repair gene mutations to explain the hypermutated phenotype. This is the first report of intramedullary spinal metastasis from a histone-mutated midline glioma. The underlying genetic alterations driving distant metastasis may involve MYCN oncogene activation as well as activation of a hypermutated phenotype potentially related to sequelae of enhanced mutated H3F3A activity.

PDTM-36. NEW THERAPEUTIC APPROACH FOR BRAINSTEM GLIOMA: INTRANASAL DELIVERY OF NANOLIPOSOMAL SN-38

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INTRODUCTION: Children with diffuse intrinsic pontine gliomas (DIPGs) die within 2 years after initial diagnosis. The infiltrative nature and anatomic location of DIPGs preclude surgical resection, and the blood-brain barrier (BBB) reduces the availability of systemically administered agents. New drug delivery approaches circumventing the BBB are greatly needed. Intranasal delivery (IND) is a practical, noninvasive method to deliver therapeutic agents into the brain along with the olfactory and trigeminal nerves pathway. With the advantages of reducing systemic side effects and convenient self-administration for patients, IND is an alternative to systemic and direct invasive drug deliveries. METHODS: Human DIPG cell lines were treated with hydrophobic fluorophore (DiI)–labeledliposomes containing SN38 (LS-SN38). Cell viability was determined by MTS assay and intracellular localization was imaged by confocal microscopy. Mice bearing human brainstem gliomas were randomly assigned to 2 groups: empty liposomes (LS-empty) and LS-SN38, administrated IND for 3 weeks. Tumor growth and response to therapy were quantitatively measured by bioluminescence imaging, and efficacy was assessed by survival analysis. *Ex vivo* distribution of fluorescent liposomes were confirmed with fluorescent microscopy. Pharmacokinetics of LS-SN38 was determined in DIPG tumor by HPLC method. RESULTS: Intracellular fluorescence signals were detected at 30 minutes and peaked at 24 hours. LS-SN38 showed greater inhibition than LS-CPT11 of DIPG cell growth. IND of LS-SN38 showed significant reduction of growth rate and prolongation of survival in compared to control group. *Ex vivo* fluorescent signals were detected throughout different brain regions, indicates diffuse distribution in brainstem. Results from pharmacokinetics will be reported at the meeting.

PDTM-37. THE ROLE OF EXOSOME miRNA DURING THE PROGRESSION OF MEDULLOBLASTOMA

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Medulloblastoma (MB) is the most common malignant paediatric brain tumor. Activation of the RAS/MAPK signaling pathway in some Shh subtype of MBs may induce target drug resistance and tumor progression. But the mechanism is not yet clear. Many studies show that exosomes carried miRNAs have close relationship with tumor invasion. The aim of present study is to clarify the relationship between exosome miRNA and RAS/MAPK signaling pathway during the progression of MBs. We performed miRNA sequencing by Ion Proton technology to analyze the miRNAs expression level between MB cells with different invasion ability. Dozens of miRNAs were identified with different expression level. Among them, 11 miRNAs were the most significant. Further gene function (GO) and signaling pathways (KEGG) analysis showed that 7 of 11 miRNAs participate in RAS/MAPK signal pathways, including miR - 221 and miR – 7, with SNCA as their candidate target gene. It was known that SNCA can interact with MAPK pathways and reduce their activation and induce cell death. It encodes the protein, a-synuclein, a well-known biomarker in PD patients. We demonstrated that expression of SNCA is rather low in Shh subtype of MB based on Nanostring Assay. Our data showed that SNCA acts as a tumor suppressor by inhibiting MB invasion and inducing cell apoptosis. In summary, exosome miRNAs of MB cells have close relationship with RAS/MAPK signaling pathway during the progression of MBs. SNCA may play a critical role in this process as a tumor suppressor. Exosome miRNAs may also be involved in regulating SNCA and be the candidate biomarkers for estimating the early recurrent and metastasis of MBs. Further investigations are needed to further clarify the interactions between a-synuclein and RAS/MAPK signal pathways, as well as to evaluate the availability of miRNA being the noninvasive biomarker for tumor invasion.

PDTM-38. PEDIATRIC MENINGIOMAS ARE CHARACTERIZED BY DISTINCT METHYLATION PROFILES DIFFERENT FROM ADULT MENINGIOMAS

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In contrast to adulthood, meningiomas are rare among children and adolescents. However, the molecular relations between both groups have not been elucidated in detail. We have analyzed 41 tumor samples from 37 pediatric meningioma patients (female: 17, male: 20; age range: 1–21 years). Atypical meningioma WHO grade II was the most frequent histological subtype (N=14, 38%). Most tumors were located at the convexity (N=18) or the skull base (N=15). Lack of *SMO*, *AKT*, *KLF4/TRAF7* mutation in Sanger sequencing (n=22) prompted whole genome sequencing of a subset (n=7). All cases exhibited bi-allelic mutation of *NF2* (combined large deletion and germline (5/7) or somatic (2/7) base exchanges/frameshifts). Subsequently, representative samples of all 37 patients were subjected to 450K DNA methylation profiling and remaining DNA to sequencing of a brain tumor specific gene panel. Loss of chromosome 22 was frequently detected (N=28, 76%), followed by loss of chromosome 1 (N=12, 32%) and chromosome 18 (N=7, 19%). Moreover, a separation into three groups was evident: One group covering all clear-cell meningiomas with enrichment for *SMARCE1* mutations, a second group dominated by atypical meningiomas, and a third group covering benign WHO grade I meningiomas, as well as rare subtypes such as rhabdoid meningiomas. Compared to adult tumors, the majority of pediatric meningiomas clustered in a separate group both by unsupervised hierarchical and clustering and t-stochastic nearest neighbor embedding. Analysis of four tumor recurrences did not reveal changes compared to the primary tumor. These data suggest that pediatric meningiomas are fundamentally different from adult counterparts.

PDTM-39. HISTONE H3 MUTATION EFFECTS ON CHROMATIN STRUCTURE AND REGULATION OF GENE TRANSCRIPTION IN PEDIATRIC GLIOMA

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INTRODUCTION: Pediatric high-grade glioma (HGG), including H3K27M diffuse midline glioma, has the highest mortality of pediatric solid tumors. Recurrent Histone H3 mutations result in methionine for lysine substitution (H3K27M) in 80% of diffuse midline gliomas, or valine for glycine/arginine substitution (G34V/R) in 50% of hemispheric HGGs. These mutations alter chromatin structure and are associated with distinct patterns of gene expression and poorer response to therapy. To elucidate the mechanism by which these mutations affect chromatin function, we characterized genomic deposition of Histone H3 proteoforms in a large cohort of rare pediatric glioma cell lines. METHODS: H3K27M DIPG (n=6), H3G34V (n=1) and wild-type pediatric high-grade glioma cells (n=2), neural stem cells (n=1) and astrocytes (n=1) were analyzed for genomic deposition patterns of H3.3, H3K27M, H3G34V, H3K27me3, H3K27Ac, H4Kme1, and H4Kme3. Extracted chromatins were sonicated to produce DNA fragments for ChIP. RNA was extracted for whole transcriptome profiling. DNA/RNA libraries were prepared using the KAPA HTP Library Preparation Kit and sequenced (ChIP-and RNA-Seq, Illumina NextSeq 500). Genomic enrichments and gene expressions were determined, quantified, and analyzed for biological relevance. RESULTS: Distinct genomic enrichment of H3 proteoforms was observed between mutant and wild type cell lines, corresponding with respective gene expression levels. Differential co-localization of H3 post-translational marks with mutant vs. wild type H3 protein was also observed, consistent with our prior description of mutant heterotypic nucleosomes. Location of specific marks (promoter, gene body) suggests altered transcription factor recruitment and function may result in observed patterns of gene expression. CONCLUSION: We present the largest known epigenetic analysis of pediatric glioma cell lines to date, indicating distinct patterns of Histone H3 enrichment and gene expression in H3K27M and H3G34V/R mutant lines. These data provide insight into the mechanisms by which H3 mutations impact pediatric glioma biology, which may inform novel therapeutic approaches.

PDTM-41. SUPER ELONGATION COMPLEX-MEDIATED TRANSCRIPTIONAL DEPENDENCY IN H3K27M-MUTANT DIFFUSE MIDLINE GLIOMAS

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Mutations in the histone 3 (H3) gene (H3K27M) are the eponymous driver in diffuse midline gliomas (DMGs), aggressive pediatric brain tumors for which no curative therapy currently exists. Emerging understanding of H3K27M biology suggests that secondary factors are required to promote oncogenesis. In order to identify specific epigenetic co-operators, we performed an shRNA screen targeting 408 genes classified as epigenetic/chromatin-associated molecules in patient-derived DMG cell lines. This identified AFF4, a component of the super elongation complex (SEC), as critical for DMG viability. We hypothesized that AFF4 promotes DMG tumorigenesis by co-operating with the H3K27M mutation to suppress scheduled transcription of pro-differentiation pathways and promote self-renewal of tumor stem cells. We found that AFF4 expression is consistently elevated in both DMG patient samples and established cell lines relative to the normal pediatric pons. We interrogated the role of AFF4 in H3K27M-mutant DMG using an shRNA lentiviral approach. Using live cell imaging, we demonstrate a significant decrease in *in vitro* clonogenicity and stem cell maintenance following AFF4 depletion. We employed RNA-seq-based gene set enrichment analysis to delineate downstream transcriptional changes under AFF4 regulatory control. Finally, we sought to determine whether CDK9, the catalytic subunit of the SEC, represents a therapeutic vulnerability in DMG. Using a combination of CDK9 overexpression and pharmacologic inhibition, we demonstrate that the disordered regulatory input of the SEC in DMG is dependent on the kinase activity of CDK9 and that this may be exploited through small molecule inhibition in both *in vitro* and *in vivo* patient-derived xenograft models. These studies represent the first pre-clinical validation of SEC inhibition as a novel therapeutic approach in pediatric DMG.

PDTM-42. TARGETED INHIBITION OF BET BROMODOMAIN AND JMJD3 PROTEINS FOR THE TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA

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Recent discovery of somatic histone gene mutations, resulting in replacement of lysine 27 by methionine (K27M) in the encoded histone H3.3 proteins, in diffuse intrinsic pontine glioma (DIPG) has dramatically improved our understanding of disease pathogenesis, and stimulated the development of novel therapeutic approaches targeting epigenetic regulators for disease treatment. K27M mutant DIPG shows a dramatic reduction in global methylation at K27 residues. We have shown that the JMJD3 demethylase inhibitor, GSKJ4, acted to restore K27 methylation in DIPG cells, while demonstrating potent anti-tumor activity, in vitro and in vivo. In addition to H3K27 methylation, H3K27 can also be acetylated (K27ac), which requires bromo- and extra-terminal domain (BET) protein activity. Increase level of H3K27 acetylation and bromodomain proteins in K27M-containing nucleosomes suggests that inhibitor of BET bromodomain protein 4 (BRD4), JQ1, could be useful for the treatment of K27M DIPG. Our aim is to investigate the hypothesis that the combined JQ1 + GSKJ4 have greater anti-tumor activity in vitro and in vivo than either monotherapy and reduce the likelihood of drug resistance. The level of H3K27 methylation and acetylation in DIPG cells with treated JQ1 + GSKJ4 were studied by western blotting. JQ1 + GSKJ4 increased H3K27 methylation and reduced K27acetylation in DIPG cells. MTS assay showed that combination treatment of JQ1 + GSKJ4 significantly increased growth inhibition, compared with either therapy alone. Colony formation assay showed that combination treatment of JQ1 + GSKJ4 significantly reduced the number of colonies. Boyden chamber assay showed that combination treatment of JQ1 + GSKJ4 significantly reduced cell invasion. Annexin V assay showed that combination treatment of JQ1 + GSKJ4 significantly promoted cell apoptosis. In vivo response to monotherapy and combination

of JQ1 + GSKJ4 will be measured by bioluminescence imaging and animal survival studies in our human DIPG xenograft model.

PDTM-43. THE ROLE OF TUMOR ASSOCIATED MACROPHAGES IN PEDIATRIC HIGH-GRADE GLIOMA

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Pediatric high-grade gliomas (pHGG) account for the most cancer-related deaths in children under the age of 19 years old. Recent advances have demonstrated that pHGGs drastically differ from their adult counterparts in terms of genetic and epigenetic alterations, suggesting they may also differ in the constituency of their tumor microenvironment. It is now known tumor associated macrophages (TAMs) can make up 30–40% of the total tumor cell mass in adult high-grade gliomas and play important roles in immune suppression and tumor promotion. This raises the question of whether pHGGs possess a distinct constituency of TAMs due to their unique genetic and epigenetic landscapes. To uncover the composition and behavior of TAMs in pHGG we utilize RCAS/tva, a somatic cell-type specific gene transfer system in which we administer RCAS-PDGFA or RCAS-PDGFB to simulate PDGF receptor activation in immune-competent new-born mice. We found that PDGFB-driven tumors have a significantly lower median survival compared to PDGFA-driven tumors. PDGFB-driven tumors also have increased infiltration of TAMs, made evident by immunohistochemical staining for IBA1. Flow cytometry analysis indicates the increased number of TAMs is due to an increased inflammatory monocyte population, but not due to the microglial population. We hypothesize these findings are attributable to the additional activation of the stromal population by PDGFB, as it can activate both PDGFRα and PDGFRβ while PDGFA only activates PDGFRα. Gene expression analysis indicates that several chemokines, chemokine receptors, macrophage, and immune markers are differentially expressed between PDGFB and -A tumor types. To establish correlations between PDGF signaling and TAM infiltration in human pediatric tumor samples, we stained 37 tumors for IBA1, PDGF-receptors, and PDGF-ligands. A positive correlation exists between high PDGFRb and PDGFB staining with high IBA1. Further studies will be done to discern the effects of increased TAM infiltration and the mechanisms driving enhanced tumor malignancy.

PDTM-44. INTRACRANIAL EPENDYMOMA: DEVELOPING PRECLINICAL MODELS AND IDENTIFYING NOVEL TREATMENTS

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INTRODUCTION: Clinical trials have not identified any effective chemotherapeutic treatments for intracranial ependymomas. This failure is partially explained by the recent identification of 6 molecularly distinct intracranial subgroups. Whilst subgroup specific preclinical investigations and treatments are required, these investigations have been hampered by the rarity of the tumours, failure to identify driver mutations for 5 of the subgroups, and difficulties establishing appropriate models. This study aims to establish patient derived cell lines, brain slice co-culture models and patient derived mouse models of intracranial ependymomas and utilise these models to identify and test novel subgroup specific drug treatments. METHODS/RESULTS: Two patient-derived intracranial ependymoma cultures have been successfully established in neurobasal and serum free media, as adherent cells and neurospheres. Whole genome methylation sequencing and immunohistochemistry are being used to characterise the cultures. Brain slice co-culture models have been successfully created, with tumour cells growing on 200μM slices of brain from neo-natal mice. Cells dissociated from patient tumour tissue and matched low passage cultures have been injected intracranially into 25 mice, with evidence of tumours in three of the mice induced with cells from the first patient tumour. A screen of 111 approved drugs on ependymoma suspension cultures, has identified 4 drugs, including Bortezomib and Ponatinib, for further investigation in the established models. CONCLUSION: Patient-derived ependymoma cells can be successfully cultured as adherent cells, neurospheres, and in co-culture models. Subgrouping the samples, screening them against approved drugs, and testing potential drugs in patient-derived models is an effective mechanism for identifying candidate drugs for subgroups of these rare tumours.

PDTM-45. POSITIVE MODULATION OF NATIVE $GABA_A$ RECEPTORS IN MEDULLOBLASTOMA CANCER CELLS WITH BENZODIAZEPINES INDUCES RAPID MITOCHONDRIAL FRAGMENTATION AND TP53-DEPENDENT, CELL CYCLE-INDEPENDENT APOPTOSIS

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Medulloblastoma is the most common childhood malignant brain tumor. Children with highest morbidity have tumors that are *TP53* wild-type and express high levels of *MYC* and *GABRA5*, which codes for the α-5 subunit of the ligand (chloride) gated $GABA_A$ receptor. Previously, we have established that the α-5 subunit of the $GABA_A$ receptor contributes to the assembly of a functional receptor in a subset of medulloblastoma cells and benzodiazepines, synthesized to function as α-5 selective ligands with psychotropic activity, impair viability[1,2]. Utilizing a *TP53* wild-type medulloblastoma cell line with amplified *MYC*, high *GABRA5* expression, and a functional α-5 $GABA_A$ receptor, we provide insight into how benzodiazepines impair cell viability by positively modulating the native $GABA_A$ receptors. We screened benzodiazepines to assess impact of varying chemical group identity on cell viability. The most potent benzodiazepine binds with specificity to ~1000 native receptors per cell with EC50 and IC50 values of ~0.8 micromolar. This binding evokes a 2 x 10^9 ions.sec-1 chloride flux, which morphologically elicits mitochondrial fragmentation, nuclei distention, and cellular blebbing. The cascade of events culminates in a caspase-mediated activation of apoptosis through the intrinsic pathway and a localization of pro-apoptotic Bcl-2-associated death promoter (BAD) protein. Benzodiazepines may be efficacious as anti-cancer therapeutics for medulloblastoma patients exhibiting a $GABA_A$ receptor expression signature by driving a chloride imbalance that leads to cell apoptosis. References: [1]Sengupta, et al. α5-GABAA receptors negatively regulate MYC-amplified medulloblastoma growth. Acta Neuropathol. 2014; 127(4): 593–603. [2]Jonas, et al. First in vivo testing of compounds targeting Group 3 medulloblastomas using an implantable microdevice as a new paradigm for drug development. J. Biomed. Nanotechnol. 2016; 12(6): 1297–1302.

PDTM-46. POLIOVIRUS RECEPTOR (CD155) EXPRESSION IN PEDIATRIC BRAIN TUMORS MEDIATES ONCOLYSIS OF MEDULLOBLASTOMA AND PLEOMORPHIC XANTHOASTROCYTOMA

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INTRODUCTION: Poliovirus oncolytic viral immunotherapy is a putatively novel approach to treat both low grade and malignant pediatric brain tumors. However, the expression of the poliovirus receptor (PVR), CD155, on a variety of pediatric brain tumors and its ability to infect, propagate, and lyse pediatric brain tumor cells is unknown. METHODS: CD155 expression in a variety of pediatric tumor specimens including pleomorphic xanthoastrocytoma (PXA), medulloblastoma, atypical teratoid rhabdoid tumor, embryonal tumor, and anaplastic ependymoma was assessed using a validated rabbit monoclonal antibody. The ability of poliovirus:rhinovirus genetic recombinant, PVSRIPO, to infect PXA (645 [BRAF V600E mutation] and 2363) and medulloblastoma (D283, D341) cell lines was determined by measurement of viral propagation and cell killing. Gene expression data from a medulloblastoma patient cohort of 763 patients was used to determine differential *PVR* mRNA expression and compared using analysis of variance. RESULTS: CD155 expression was present in 53 of 57 patient specimens analyzed in all PXA and medulloblastoma cell lines. One-step growth curves of PVSRIPO at a multiplicity of infection of 10 demonstrated productive infection and peak plaque formation units at 5–10 hours. PVSRIPO infection of all four cell lines demonstrated decreased proliferation in 2363, 645, and D341 cell lines at 48 hours ($p<0.001$) and resulted in cell death. *PVR* expression was significantly highest in Group 3γ, WNTα, and WNT (subgroups with c-myc overexpression) compared to the other medulloblastoma subtypes ($p<0.001$). CONCLUSIONS: Poliovirus receptor, CD155, is widely expressed in a variety of brain tumor specimens. This proof-of-concept *in vitro* study demonstrated that PVSRIPO was capable of infecting, propagating, and prohibiting cell proliferation in PXA with and without BRAF V300E mutations and Group 3 medulloblastoma. Future studies will evaluate PVSRIPO to treat pediatric brain tumors and the possible link between c-myc and PVR expression.

PDTM-47. REAL TIME IN VIVO MONITORING OF 18F-LABELED PANOBINOSTAT PHARMAKOKINETICS FOR TREATMENT OF DIFFUSE INTRINSIC PONTINE GLIOMA (DIPG) VIA CONVECTION ENHANCED DELIVERY (CED)

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Diffuse intrinsic pontine glioma (DIPG) is a universally fatal tumor of childhood with median survival of one year: an initially good response to radiation therapy is invariably followed by recurrence. Recent promise comes from the use of histone deacetylase complex (HDAC) inhibitors which normalize the aberrant epigenetic environment of DIPG. Of these, the pan-HDAC inhibitor panobinostat has been rapidly translated into early phase clinical trials. The drug, however, poorly penetrates the blood-brain barrier (BBB) and thus might be better administered by convection enhanced delivery (CED). To more clearly define pharmacokinetics and therapeutic trial design, compounds with both imaging and therapeutic features (i.e. theranostics) would be advantageous. Here, we modify panobinostat to include a trifluoroborate [^{18}F]F_2B- moiety that makes it visible with positron emission tomography (PET). We demonstrated that this modified compound retains its bioactivity against glioma cell lines (IC50 of 91.49 nM and 94.94 nM against SU-DIPG-IV and SU-DIPG-XIII cell lines) and proteomics profile. This novel theranostic compound, PETobinostat, when administered via CED in the rodent brain stem demonstrates a half-life of 126 (95% CI: 109.6–146.9) minutes using decay-corrected dynamic PET scanning. The significant mouse-to-mouse variability highlights the importance of individualized patient imaging of drug delivery. Comparatively, systemic administration (intravenous or intraperitoneal) show poor cranial accumulation (39.3%, 1.5%, and 0.44% for CED, IV, and IP respectively). Both in vivo PET scanning and post mortem scintillation biodistribution elucidate routes and rates of biological PETobinostat clearance following CED. Treatment of tumor-bearing animals with both PETobinostat or the unmodified panobinostat shows significant tumor regression (2.3 fold diffrence) when compared to vehicle. Furthermore, PETobinostat allowed monitoring of tumor permeation and clearance following CED. We believe that this compound holds promise in helping us establish a CED-based panobinostat/PETobinostat clinical trial, where real-time information on pharmacokinetics and pharmacodynamics allows us to maximize therapeutic efficacy during CED.

QUALITY OF LIFE AND PALLIATIVE CARE

QOLP-01. EFFECTS OF TUMOR TREATING FIELDS ON HEALTH-RELATED QUALITY OF LIFE (HRQoL) IN NEWLY DIAGNOSED GLIOBLASTOMA: AN EXPLORATORY ANALYSIS OF THE EF-14 RANDOMIZED PHASE III TRIAL

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INTRODUCTION: Tumor Treating Fields (TTFields) are a novel treatment modality that continuously delivers alternating electric fields to the tumor region. TTFields interfere with the assembly of the mitotic spindle leading to apoptosis. In the EF-14 phase III study in newly diagnosed glioblastoma, TTFields plus temozolomide (TTFields/TMZ) showed significant increase in overall and 5-year survival rates compared to temozolomide. TTFields/TMZ did not negatively impact nine prespecified HRQoL scales (global health, physical, cognitive, role, social and emotional functioning, itchy skin, pain, and leg weakness) except for increased itchy skin. We present an exploratory analysis of the remaining 17 EORTC QLQ C-30 and BN-20 HRQoL scales. METHODS: HRQoL was measured by the EORTC QLQ-C30 and BN20 questionnaires at baseline and every 3 months thereafter. Mean changes from baseline as well as significant changes in scores over time (>10 points) were evaluated using a repeated measures test. Deterioration-free survival and time-to-deterioration in HRQoL were assessed for each scale, as well as % patients with stable/improved HRQoL versus baseline. RESULTS: No statistically or clinically significant decline in any of the exploratory HRQoL scales was seen in the repeated measures analysis or in time to deterioration. Significantly more patients treated with TTFields/TMZ versus TMZ reported stable/improved bladder control (63.6% versus 46.8%, p=0.001) and diarrhea (60.6% versus 43.7%, p=0.001) compared to baseline. The deterioration-free survival for diarrhea, future uncertainty and headaches was significantly delayed in TTFields/TMZ treated patients compared to TMZ alone (HR 0.68, 0.71 and 0.67, respectively, p<0.001). CONCLUSIONS: No negative impact of HRQoL was seen in any of the exploratory scales between patients treated with temozolomide only or TTFields and TMZ. Adding TTFields to standard therapy in newly diagnosed glioblastoma does not appear to negatively impact QoL.

QOLP-02. INSURANCE STATUS IMPACTS THE ECONOMIC BURDEN AND SURVIVAL OF GLIOBLASTOMA PATIENTS WITH HEALTH INSURANCE

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INTRODUCTION: Glioblastoma carries a high economic burden for patients and caregivers. We investigated the hospital costs of surgery for newly-diagnosed glioblastoma patients based on insurance status. METHODS: Retrospective review of GBM patients undergoing first resection at UCSF and corresponding hospital charges from 2010–2015. RESULTS: Of 227 patients (median age= 62; females= 37.9%), 31 (13.7%) had Medicaid, 94 (41.4%) had Medicare, and 102 (44.9%) had private insurance. Medicaid patients had 30% higher mean overall hospital costs for resecting GBM compared to non-Medicaid insurance patients ($50,285 vs $38,800; p= 0.015). Sub-cost analysis revealed Medicaid patients had higher ICU, OR and imaging costs versus non-Medicaid insured patients ($13,400/$16,470/$2,182 vs $9,700/$14,770/$1,357; p= 0.01/p= 0.03/p< 0.0001). Kaplan-Meier survival analysis showed Medicaid patients had the shortest overall survival (10.7 months, Medicare = 12.8 months, Private insurance = 15.8 months; p=0.02). Tumor diameter at diagnosis was largest for Medicaid (4.7 cm) versus Medicare (4.1 cm) and privately insured patients (4.2 cm; p=0.03). Only 67.74% of Medicaid patients had PCPs versus 91.5% and 86.27% of Medicare and privately insured patients, respectively (p=0.004), at their initial visit to our institution. Medicaid patients had longer overall and ICU lengths of stay (6.9 and 2.6 days) versus Medicare (4.0 and 1.5 days) and privately insured (3.9 and 1.8 days; p < 0.01) cohorts. Moreover, Medicaid patients had similar comorbidity rates as Medicare patients (67.8% vs. 67.18%), but both groups had higher comorbidity rates than privately insured patients (40.4%; p< 0.0001). CONCLUSIONS: Despite higher surgical costs and longer lengths of stay, GBM patients with Medicaid have poorer survival. This may reflect that these patients lacking PCPs and, thus, having more comorbidities and presenting later in the disease course with larger tumors consume more hospital resources such as OR time and confer increased operative risk.

QOLP-03. END OF LIFE PHASE IN GLIOBLASTOMA: PROSPECTIVE ASSESSMENT OF SYMPTOM BURDEN AND SYMPTOM INTERFERENCE IN THE END OF LIFE PHASE- FINAL ANALYSIS

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PURPOSE: To prospectively study symptoms and quality of life in terminal stage Glioblastoma (GBM) patients. End-of-life (EoL) symptoms of patients with GBM are poorly understood and have not been prospectively assessed in larger outpatient cohorts. The aim of this study was to assess prominent EoL symptoms in GBM to guide future treatment decisions and palliative care guidelines. METHODS: Prospective single-center study assessing symptoms and QoL of GBM patients with low performance status (Karnofsky Performance Status 3rd line therapy with bevacizumab and showing clinical and radiological progression. The MD Anderson Symptom Inventory Brain Tumor Module and neurological assessments were used upon enrollment and during bi-weekly phone calls with patients and caregivers. Mixed models were used to assess changes over time for symptoms and interferences over the last 14 weeks prior to death. RESULTS: Data from 50 patient-caregiver dyads was available for interpretation. The highest reported symptoms during the EoL phase with significant progression (p<0.01) were: Drowsiness, concentration, fatigue, speaking and understanding. High symptoms that did not progress significantly were remembering and weakness. 24% of all patients reported seizures, but the overall reported impact on symptom burden was low and did not increase in the EoL phase. Pain and GI symptoms were rated low and without significant progression. Interference scores for activity, work, relations, walking, enjoyment of life and decision making were high and increased significantly during the EoL phase. CONCLUSION: This is the final analysis of a large prospective study assessing EoL symptoms in GBM outpatients. Patients have severe symptom burden affecting decision making in the last weeks of life. Traditional cancer symptoms such as nausea, vomiting and pain were low. Palliative care should focus on early advance care planning and decision making.

QOLP-04. THE EVOLVING ROLE OF COMPLEMENTARY CANNABIS THERAPY IN GLIOBLASTOMA TREATMENT

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BACKGROUND: Evidence suggests a therapeutic role for cannabinoids, including cannabidiol (CBD) and tetrahydrocannabinol (THC), to treat glioblastoma (GBM) in cell cultures and animal models. It is unknown if cannabinoids can effectively treat GBM in human patients. METHODS: We define therapeutic treatment as ingesting a cannabis oil concentrate of at least 50 mg of cannabinoid per day, for at least one month, while also receiving standard-of-care treatments. In Part 1 of our analysis, two time periods were defined. During Time Period 1 (TP1, 9/1/2014–6/1/2017), 12 GBM patients registered for CT MMP. During Time Period 2 (TP2, 6/1/2017-6/1/2018), 11 GBM patients registered for CT MMP. Data includes patient age, gender, ethnicity, tumor characteristics, cannabis treatment strategy (low use, palliative, or therapeutic), when the patient initiated cannabis relative to the diagnosis date, and overall survival (OS). In Part 2, we evaluated

OS at 1 and 2 years in patients who used therapeutic cannabis, compared to those who had low or palliative use. RESULTS: During TP1, 6 patients used therapeutic cannabis. 5 patients are still alive (median of 25.4 months from diagnosis). 7 patients were male, with median age of 53 (range 17–74). During TP2, 9 patients used therapeutic cannabis. 6 patients are still alive (median of 8.8 months from diagnosis). 9 patients were female, with median age of 65 (range 50–78). Among the 23 patients who registered for CT MMP, therapeutic cannabis use was associated with increased OS at 1 year (80% vs 74%, p=0.03), and increased OS at 2 years (73% vs 65%, p=0.002) compared to low or palliative use. Therapeutic cannabis therapy was well tolerated, with no significant adverse events. CONCLUSIONS: Therapeutic cannabis therapy can be integrated into standard-of-care treatments for GBM. Our data suggest that there may be a survival benefit. Further research studies of cannabis are warranted.

QOLP-05. THE EFFECT OF RESILIENCE ON QUALITY OF LIFE IN PATIENTS WITH HIGH GRADE GLIOMAS AND THEIR CAREGIVERS

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Quality of life is often impaired in patients with high grade gliomas (HGG) and their caregivers. Resilience refers to one's ability to maintain or improve their emotional wellbeing in the setting of extreme stressors. In patients with chronic illnesses including cancer, higher levels of resilience are correlated with improved quality of life. This has not been well studied in HGG patients or their caregivers. We designed a prospective cohort study to assess this relationship in 50 patient-caregiver dyads. All patients were diagnosed with HGG in the past 6 months. Caregivers were self-identified by patients as the primary person providing physical or emotional support since diagnosis. Resilience was assessed using the Connor Davidson Resilience Scale (CD-RISC) in patients and caregivers. Quality of life was assessed using the EORTC-QLQ30 and BN-20 scales in patients and the Caregiver Quality of Life Cancer scale (CQOLC) in caregivers. After obtaining informed consent, demographic information was collected and participants completed the CD-RISC and QOL surveys. IRB approval was granted in May 2018 and in the 3 weeks following approval, 15 dyads have been consented and 3 have completed surveys. To date, median age of both patients and caregivers is 55.6 years. All patients carry a diagnosis of glioblastoma and all caregivers identify as spouses or long-term partners. Caregivers report impaired QOL with an average score of 67 out of a possible 140 points (higher scores indicating better QOL). Median global quality of life score in patients is 80 which is higher than previously reported medians. There are currently too few patients to draw correlations; however, based on the current rate of participation, we anticipate accrual will complete within 5 months. We will present the updated data. If positively correlated, interventions targeting resilience may also improve quality of life in this population.

QOLP-06. BODY IMAGE DISTURBANCE IN PATIENTS WITH PRIMARY BRAIN TUMORS

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BACKGROUND: Perception of body image is an important issue for cancer patients, as patients with body image concerns are susceptible to psychosocial consequences such as depression, anxiety, difficulty coping, and poor quality of life (QoL). While this concern has been documented in patients with similar heightened risk due to visible disfigurement and lifestyle alterations, such as in head and neck tumors, currently no data exists of this QoL issue in patients with primary brain tumors (PBT). METHODS: A cross-sectional survey of 100 PBT patients was conducted as part of an IRB approved prospective protocol using structured questionnaires. Participants completed the 10-item Body Image Scale (BIS) questionnaire to assess the prevalence of body image disturbance. Clinically significant body image disturbance was defined as a BIS score ≥ 10. Cronbach's alpha was used to assess reliability. RESULTS: The median age was 48 (range 23–74), 86% were Caucasian, and 56% were male. Glioblastoma was the most common diagnosis (32%), with low grade tumors (I-II) representing 30% of the sample. Median time from initial diagnosis was 5 years (range 0–22), and 64% of patients had a KPS of ≥ 90. Overall, the median BIS score was 5 (range 0–27). The prevalence of clinically significant body image disturbance was 28% (95%CI: 19%-37%). Cronbach's alpha for the BIS in this population was 0.91, with all 10 items contributing to its reliability. CONCLUSIONS: This is the first study to explore altered body image in PBT patients. Clinically significant body image disturbance was present in nearly 1/3 of patients, and was similar to other solid tumor patients felt to be at heightened risk. The BIS demonstrated good internal consistency. This data supports future research into the characteristics and etiology of this QoL issue among PBT patients.

QOLP-07. QUALITY OF LIFE IN PATIENTS WITH MENINGIOMA

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OBJECTIVE: Meningiomas are the most common primary central nervous system tumor, and patients typically live for many years after diagnosis and treatment. Traditionally, patient outcomes are recorded from a clinician's standpoint, including extent of resection, surgical/radiation complications, progression-free survival, and tumor recurrence. Outcomes from this standpoint will underestimate the impact of disease and treatment on a patient's quality of life. Thus, we have started a multi-institutional prospective study to assess the quality of life in patients with meningioma. METHODS: A quality of life instrument (QoL-MNG) was developed using questions from the Functional Assessment of Cancer Treatment-Meningioma (FACT-MNG) tumor site specific instrument on a web-based platform. A pre-operative baseline questionnaire is collected for all patients. Subsequently, patients are assigned to treatment (surgery or radiation) or observation groups. All patients complete a baseline questionnaire. The treatment group completes questionnaires at 3-, 6-, 12-, 18-month, and then annually following surgery or radiation. The observation group completes annual questionnaires. Clinical outcomes data are collected at each time point. RESULTS: To date, 55 patients from a single-institution are actively enrolled and have completed baseline questionnaires in the QoL-MNG study using the online-based interface. Approval for the study has been obtained at 18 other sites. We will present patient level data from our first 14 patients who have completed follow up questionnaires. This includes outcomes in physical well-being, social/family well-being, emotional well-being, functional well-being and tumor site specific questions in the following tumor locations: cerebellopontine angle, parasagittal, petroclival, sphenoid wing, and convexity. CONCLUSIONS: Assessing quality of life is fundamental to improving care for patients with meningioma. These are the first preliminary results of our prospective study which we have expanded to eighteen institutions across North America and Europe with the goal of improving our understanding the impact of meningioma on quality of life.

QOLP-08. A NEURO-ONCOLOGY CAREGIVER SUPPORT GROUP, AN EFFECTIVE WAY TO PROVIDE EMOTIONAL SUPPORT FOR CAREGIVERS

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BACKGROUND: Brain tumor support groups provide education and emotional support to patients and families dealing with a brain tumor diagnosis. These groups are beneficial, but at times caregivers report that they must hold back and not speak their truth/reality for fear of hurting the patients feelings. A neuro-oncology caregiver support group is one easy and economical way to provide caregivers with emotional support and foster resilience. A regular attendee reminds us that caregivers need a place to not have to be strong, to not have to be everything all the time, and to be around people that understand. METHOD: A UCSF neuro-oncology caregiver support group is led by a social worker and neuro-oncology RN once a month. The group provides caregivers a safe space to share feelings and focus on their experience of being a caregiver and coping with the emotional aspects of caring for someone with a brain tumor. Participants are invited to acknowledge and share the complicated emotions that they experience. Themes are documented and participants surveyed annually. RESULTS: In 2017–2018, 30 caregivers attended the monthly meetings with an average of 10 attendees. 6 attended all meetings in the last year. Common themes include sorrow, anger, ambiguous loss, guilt, and ways to find joy and humor. Annual survey results demonstrate a mix of male and females, some who travel over 75 miles to be at the meeting. 93% of those responding reported benefit from the opportunity to normalize feelings and learn from each other. 93% reported that the group reduced their sense of isolation and 100% rated the group as beneficial and would recommend the group to others. CONCLUSION: A neuro-oncology caregiver specific support group is

a feasible and economic way to assist caregivers in normalizing complicated emotions, connecting with similar others, and may foster resilience.

QOLP-09. ASSOCIATION BETWEEN BODY IMAGE INVESTMENT AND ALTERATION IN PATIENTS WITH PRIMARY BRAIN TUMORS

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BACKGROUND: Body image is an important psychosocial issue in cancer patients, including those with primary brain tumors (PBT). Investment in physical appearance is associated with depression and quality-of-life (QoL) outcomes in non-CNS malignancies. Therefore, examination of body image investment is warranted to better understand factors involved in body image disturbance in the PBT population. METHODS: A cross-sectional survey of 100 PBT patients was conducted as part of an IRB approved prospective protocol. Participants completed the 10-item Body Image Scale (BIS), and the 20-item Appearance Schemas Inventory-Revised (ASI-R) which includes self-evaluative and motivational salience subscales. Potential BIS and ASI-R scores ranged from 0–30 and 1–5 respectively. Cronbach's alpha assessed reliability. Pearson's correlation coefficients assessed relationships between questionnaires. Significance level was $p< 0.05$. RESULTS: Patients were mostly male (56%) with a median age of 48 (range 23–74). Glioblastoma was the most common diagnosis (32%). Median time from initial diagnosis was 5 years (range 0–22), and 64% of patients had a KPS≥ 90. Median BIS score was 5 (range 0–27), ASI-R composite score was 2.9 (range 1.5–4.7), self-evaluative subscale score was 2.6 (range 1.2–4.5), and motivational subscale score was 3.4 (range 1.9–5.0). BIS was significantly correlated with ASI-R composite (r=0.53, 95%CI: 0.37 to 0.65), ASI-R self-evaluative (r=0.65, 95%CI: .52 to .75), but not ASI-R motivational (r=0.14, 95%CI: -0.06 to 0.33, p=0.17) scores. Increased severity of altered body image was correlated to increased negativity of the patient's belief about their appearance. Cronbach's alpha for the ASI-R composite, self-evaluative and motivational subscales were 0.87, 0.85, and 0.81 respectively. CONCLUSIONS: Body image and investment are important QoL issues for PBT patients. The ASI-R demonstrated good internal consistency in this population. While there was significant correlation between BIS and ASI-R composite and self-evaluative scores, this was not true with the motivational score.

QOLP-10. THE IMPACT OF VARIOUS FACTORS ON HEALTH-RELATED QUALITY OF LIFE (hrQOL) IN PATIENTS UNDERGOING INTRACRANIAL RADIOTHERAPY

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PURPOSE: Treatment of brain tumors significantly impacts health-related quality of life (hrQOL). In this prospective longitudinal study of hrQOL in patients receiving intracranial radiotherapy (RT) we sought to better understand the factors impacting patient hrQOL following brain RT. METHODS: Data was collected prospectively at our institution from 2013–2017. Prior to RT, patients completed a core overall hrQOL tool (EORTC QLQ C-15-PAL) and a brain tumor-specific module (QLQ-BN20), repeating these questionnaires one month post-treatment and every 3-months until patient death or study withdrawal. Baseline patient data collected included age, gender, KPS, tumor type (primary or metastasis), histology, and RT treatment type. Statistical analyses using R software package included linear regression modeling for continuous hrQOL outcomes and proportional odds modeling for ordinal hrQOL outcomes. RESULTS: Two hundred twenty one patients (51% male, 49% female) completed baseline surveys. The majority of patients had brain metastases (57%) while 43% had a primary brain tumor (13% WHO grade I-II, 26% WHO grade III-IV, and 4% benign/other). Global hrQOL was significantly affected by tumor type, histology, and RT treatment type one month post-RT, with metastatic patients 41% less likely to give a higher score than primary brain tumor patients (p=0.031), WHO grade I-II patients 8 times more likely to give a higher score than those with brain metastases (p=0.017), and patients receiving SRS or WBRT 75–81% less likely to give a higher score than those receiving partial brain RT (p=0.039). Other hrQOL measures impacted by various clinical and treatment factors included future uncertainty, emotional and physical functioning, fatigue, motor dysfunction, and nausea and vomiting. CONCLUSIONS: We demonstrated significant longitudinal overall and brain tumor-specific hrQOL differences due to such factors as tumor type, disease histology, and treatment type following intracranial RT. This knowledge may help better predict certain hrQOL outcomes and guide patient care following intracranial RT.

QOLP-11. QUALITY OF LIFE IN HIGH-GRADE GLIOMA PATIENTS ON A PHASE I VIROTHERAPY STUDY

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BACKGROUND: The therapeutic potential of virotherapy for malignant glioma has sparked much interest recently, however, little is known about its effect on quality of life (QOL). We sought to explore patients QOL before and after treatment with a neural stem cell (NSC) driven oncolytic adenovirus (CRAd-S-pk7) plus standard of care therapy using the FACT-Brain. METHODS: Patients with pathologically confirmed high-grade glioma at the time of resection received an intratumoral injection of CRAd-S-pk7. Concurrent radiotherapy to 60Gy with daily temozolomide (75mg/m2) followed, beginning within 7–10 days after surgery. Patients then received 6 cycles of adjuvant temozolomide (150–200 mg/m2) for the first 5 days every 28 days, and then were followed until progression. FACT-Brain was collected at baseline, day 14, day 56 (end of concurrent period), and then every 8 weeks during the adjuvant period. RESULTS: 7 patients have been enrolled to date, all with glioblastoma. Mean FACT-Brain Total and TOI scores at baseline were 162.0 (SD=19.1) and 115.3 (SD=23.8), consistent with expected baseline QOL scores in the newly diagnosed population. Mean scores declined significantly ($p=.05$ and $p<0.01$ respectively) by the end of concurrent treatment to 142.7 (SD=31.4) and 99.0 (SD=23.8), rebounded after adjuvant Cycle 1, and were similar to baseline by cycle 2. CONCLUSION: HGG patients treated with NSC virotherapy have declines in QOL after completing concurrent chemoradiation followed by improvement. This QOL pattern is similar to patients receiving standard treatment and may help clinicians discuss the impact of virotherapy with future patients. In addition, information may assist in evaluating net clinical benefit in future virotherapy studies.

QOLP-12. A NOVEL MULTIDISCIPLINARY CARE CLINIC MODEL FOR FRAIL PATIENTS WITH CENTRAL NERVOUS SYSTEM MALIGNANCIES

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BACKGROUND: Physical, cognitive, and existential needs are common in patients with central nervous system (CNS) malignancies, however not all needs can be addressed in routine clinic visits. To identify and address complex patient and caregiver needs, we implemented a novel multidisciplinary care clinic (MDCC) for patients with CNS malignancies at our institution. METHODS: Monthly between 8/2017-4/2018, a team comprising a neurologist, physiatrist, nurse, physical therapist, case manager, social worker, dietitian and chaplain assessed patients with CNS malignancies, neurological deficits and KPS ≤80. Within a 3 hour-visit, providers rotated in to see patient/caregiver. Starting in 11/2017, participants completed validated surveys on unmet needs, financial strain, and home equipment prior to MDCC and on satisfaction after clinic. Descriptive statistics were used to analyze results. RESULTS: Forty-two patients were seen: twenty-two (50%) were female; median age=64. Diagnoses included gliomas (n=30), brain metastases (n=8), other primary brain tumors (n=4); median KPS=70 (range 50–80). Thirty-one (74%) patients were on cancer-directed therapy and eleven (26%) on surveillance. Twenty-five patients completed pre-MDCC surveys, the predominant unmet need (96%) was: "not being able to do the things you used to do"; 15 (60%) expressed worry about their ability to pay for cancer care. Twenty-four caregivers completed the pre-MDCC survey, the predominant unmet need (83%) was: "I need my partner to have an ongoing case manager." At MDCC, 38 (90%) patients received recommendations for symptom management, 34 (81%) discussed prognosis and/or coping with cancer, and 33 (78%) established or updated their physical therapy and/or home services. Twenty-four patients (57%) completed a post-MDCC satisfaction survey: 100% felt the clinic was helpful, and 96% would recommend the clinic to others. CONCLUSION: This study identifies a new shared-appointment model of care to identify and address unmet

needs. Additional prospective study is needed to determine the role of this clinic in neuro-oncologic care.

QOLP-13. PSYCHOSOCIAL DISTRESS IN PATIENTS WITH RECURRENT MENINGIOMAS

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INTRODUCTION: Meningiomas, the most common CNS neoplasm, are often deemed less threatening than their glial counterparts. Unfortunately, these tumors recur and necessitate surgery, radiation, and/or systemic treatment. We explored the psychosocial distress of patients with recurrent meningioma using The National Comprehensive Cancer Network Distress Thermometer (NCCN-DT) and Problem List. METHODS: This was a retrospective analysis of patients with recurrent meningioma seen at the Preston Robert Tisch Brain Tumor Center between 12/31/2004–10/10/2018 who completed a NCCN-DT and Problem List. The first or only NCCN-DT assessment after initial recurrence was used for analysis with a score of 4 indicating moderate to severe distress. RESULTS: 45 patients were identified, 56% female, median age was 61 years and 58% had unifocal disease. 60% were Grade 1, 33% Grade II, 4% Grade III, and 2% indeterminate recurrent meningioma. The median NCCN-DT score was 3, and 49% had a NCCN-DT score 4 indicating moderate to severe distress. 64% of females vs 30% of males reported distress scores 4 (p=0.04). 65% of patients with unifocal disease reported 4 scores compared to 26% with multifocal disease (p=0.02). Fatigue (N=24), Worry (N=23), Nervousness (N=22), Depression (N=19) and Memory/Concentration (N=19) were the most commonly reported problems. A higher incidence of worry among females (64%) than males (35%) was the only problem showing a trend towards significance (p=0.08). Between initial recurrence and NCCN assessment, the type and number of treatments patients received included: surgery (median=1, range 0–5), radiation (median=2, range 0–5), systemic treatment (median=2, range 0–12). There was no association between distress and the number of surgeries (p=0.99), radiation treatments (p=0.49) or systemic therapies (p=0.87). CONCLUSIONS: In our study population, nearly half of recurrent meningioma patients reported moderate to severe distress. Therefore, even in this benign tumor population, the NCCN-DT and problem list should be administered at every clinic visit.

QOLP-14. PRELIMINARY EXAMINATION OF CONFIRMED GLIOMA RISK FACTORS AMONG EPENDYMOMA PATIENTS IN THE NEURO-ONCOLOGY BRANCH NATURAL HISTORY STUDY (NOB-NHS) AND RISK AND OUTCOMES STUDY (ROS)

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BACKGROUND: The Glioma International Case-Control (GICC) study is the largest study to date examining genetic and environmental risk factors for adult gliomas. Inverse associations have been repeatedly confirmed for allergies, atopic skin diseases, and viral infections. Evaluations of these risk factors specifically ependymoma has not been completed, due to the rarity compared to other glioma types. Therefore, the purpose of this report is evaluating the associations of these confirmed glioma risk factors with ependymomas. METHODS: Adult ependymoma patients (n=128) enrolled in the NOB-ROS and NHS completed a risk factor questionnaire adapted from the GICC study via a web-based portal. Survey sections related to history of asthma/allergies, common infectious diseases, and regular antihistamine/anti-inflammatory use were examined. Ependymoma patients exposed to these factors were calculated and compared to control (n=1,534) and glioma cases (n=1,339) from a published report (Scheurer, 2011). Odds ratios were calculated for ependymoma using the published controls to compare risk factor effects between ependymoma and glioma cases. RESULTS: The sample was mostly female (62%), median age=45, white (95%), diagnosed with an ependymoma (52%)in the spine (66%). Ependymoma patients were: less likely to have history of asthma/allergy (41%) than controls (66%; OR 0.36, p<0.001); more likely to report regular antihistamine use (23%) than controls (11%; OR 2.38, p<0.001) and all glioma cases combined (p<0.001); and more likely to report regular anti-inflammatory use than all glioma cases combined (p=0.02). CONCLUSION: Asthma/allergy effects may be more pronounced among ependymoma cases compared to gliomas overall. However, effects of antihistamines and NSAIDs are MUCH worse in ependymoma cases compared to published effects in all cases. This is the first report in adult ependymoma patients exploring risk factors reported in other gliomas and provides preliminary understanding of potential differences in ependymomas. Further analysis should be explored to identify significant areas of concern.

QOLP-15. SAFETY AND ADVERSE EVENT PROFILE OF TUMOR TREATING FIELDS IN ANAPLASTIC GLIOMA A POST-MARKETING SURVEILLANCE ANALYSIS

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INTRODUCTION: Tumor Treating Fields (TTFields) are approved for glioblastoma (GBM) based on two randomized phase 3 trials. The Optune system non-invasively administers TTFields at 200kHz via transducer arrays placed upon a shaved scalp. Despite advancements in the molecular characterization of anaplastic gliomas (AGs), there remains a paucity of treatment options. While Optune is not FDA-approved for AGs, there are several open clinical trials. To further characterize potential safety risks in AG, post-marketing surveillance data are reported for patients with AGs treated with TTFields. METHODS: A review of adverse events in patients with a diagnosis of anaplastic astrocytoma or anaplastic oligodendroglioma treated with TTFields. Post-market surveillance data were analyzed based on the MedDRA body system (system organ class) preferred terms. RESULTS: A total of 498 patients with AGs were treated with TTFields in the United States and Europe. Of these, 262 patients (53%) experienced at least 1 adverse event (AE). These included heat sensation in 50 (10%), electric sensation in 36 (7%), headache in 41 (8%) and fatigue in 14 (3%). Skin toxicity was the most common type of AE reported in 152 patients (31%) and included skin reaction (30%), skin ulcer (3%), hyperhidrosis (3%), and rash (<1%). These findings as well as the incidence of other reported AEs were in line with glioblastoma phase 3 trials. CONCLUSION: In this retrospective review of available post-marketing surveillance data, there were no unexpected adverse events when TTFields were used in patients with AGs. The incidence of the most common AE, skin reaction, as well as other reported AEs were comparable with rates observed in the EF-11 trial for patients with recurrent GBM, and the EF-14 trial for patients with newly diagnosed GBM. This data supports further investigation on safety and efficacy of TTFields in patients with AGs, and prospective studies are currently planned.

QOLP-16. CAPTURING THE PRIMARY BRAIN TUMOR (PBT) PATIENT'S EXPERIENCE OF BODY IMAGE DISSATISFACTION: REPORT FROM THE NEURO-ONCOLOGY BRANCH-NATURAL HISTORY STUDY (NOB-NHS)

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BACKGROUND: Body image dissatisfaction is associated with difficulty coping and adjustment in patients with other solid tumors. Although PBT patients may be at increased risk due to the visible disfigurement and debilitating nature of the disease, there is limited research in this patient group. The purpose of this report is to present the qualitative analysis of data captured on the NOB-NHS Body Image Study. METHODS: 100 patients participated by completing structured questionnaires and open-ended responses. Seventy-two patients provided open-ended feedback exploring, How have the changes affected you? within the Feedback Form. Qualitative analysis software (MAXQDA) allowed for text coding of response data to identify recurrent themes. RESULTS: The sample was primarily white (82%), males (58%) median age=50 (range 23–74), diagnosed with glioblastoma (36%). Only 10 (8%) indicated no body image issues. Five dominant themes (lifestyle changes, symptom effects, negative & positive outlook, changes in appearance) characterized participant description of body image since their diagnosis. A number of patients (28%) expressed a hindrance within their lifestyle (altered mobility, independence, activity, and changes in relationships) contributing to this theme. Participants described symptom

effects (weight gain, fatigue, pain, vision changes) contributing to dissatisfaction throughout the disease trajectory. Specific changes in appearance (hair loss, indentation on head, skin dryness) were also problematic factors affecting adjustment and coping. An ascribed negative outlook (9%) (self-conscious, feeling ashamed or vulnerable) magnified awareness of long-term permanent changes, but some (8%) described the implementation of a positive outlook (using exercise and hope) allowing for acceptance of these changes. CONCLUSION: These findings offer insight from the patients perspective on identified physical, mental and treatment-related factors regarding body image concerns and the scope of issues faced by PBT patients. Understanding patient concerns allows for a multidimensional approach in management of key areas with the goal of improving overall quality of life.

QOLP-17. REVIEW AND META-ANALYSIS OF NAUSEA AND VOMITING TRIALS FOR MALIGNANT GLIOMAS

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BACKGROUND: Nausea (N) and vomiting (V) continues to be one of the most feared side effects of individuals undergoing cancer treatment with 10–40% (or 60–90% not) experiencing NV with modern-day antiemetics. Trials establishing antiemetic guidelines excluded glioma patients. OBJECTIVES: To assess overall NV efficacy and safety across multiple glioma trials using palonosetron (PAL), aprepitant plus ondansetron, or ondansetron alone in glioma patients receiving either concomitant radiation therapy and temozolomide, adjuvant temozolomide post-chemoradiation, or irinotecan and bevacizumab in the recurrent setting. METHODS: Review of 270 patients enrolled in four Phase II glioma antiemetic trials was conducted to evaluate complete response (CR) rate defined as no vomiting or use of rescue anti-emetic on day 1 (Acute-CR), days 2–5/7 (Delayed-CR), and overall (Complete Control: CC) during 1 week of treatment. Chemotherapy-induced nausea (CIN) and vomiting (CIV) rates were defined as no nausea or no vomiting during the study period, respectively. RESULTS: Mean age=54.8, 61% male, median KPS 80%, 83% GBM. CC for patients receiving PAL with radiation and temozolomide was 74%; Acute-CR was 87%; Delayed-CR was 82%. CIN rates (6779%) were less than CIV rates (8797%). For 63 recurrent patients receiving irinotecan and bevacizumab, the CC rate was 47%, Acute-CR was 62%, and Delayed-CR was 62%. PAL with 5-day adjuvant temozolomide demonstrated a CC of 88%, Acute-CR of 94%, and Delayed-CR of 88 % in 33 patients. CR rates for the 70 patients in the aprepitant/ondansetron arm receiving adjuvant temozolomide were CC: 58.6%; Acute-CR: 97.1%; Delayed-CR: 58.6% and for the 66 in the ondansetron alone arm were CC: 54.5%; Acute-CR 87.9%; Delayed-CR 57.6%. Main attributable adverse events included constipation, headache, and diarrhea and were all grade 1–2. CONCLUSIONS: Review of NV rates in glioma patients are comparable to other cancers. Meta-analysis and safety results will be presented.

QOLP-18. PSYCHOLOGICAL INTERVENTION APPLIED TO MINIMIZING THE USE OF SEDATIVES IN 3–14 YEARS OLD CENTRAL NERVE SYSTEM TUMORS UNDERGOING RADIOTHERAPY

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OBJECTIVE: This article describes the application evaluation of psychological interventions in 3–14 years old central nerve system tumor children undergoing radiotherapy, furthermore, to promote optimal psychological interventions for local Chinese children. METHODS: Between January 2016 and November 2017, 166 children patients received radiotherapy in Department of Oncology, Guangdong 999 Brain Hospital, 53 children(33 boys and 20 girls) of them with medical fear. Therefore, the medical team conducted psychological interventions to them before radiotherapy. Specially designed psychological intervention program included: Psychological scales, medical games, psychologists participated in the whole course of radiotherapy. The nurse recorded the usage of sedatives and heart rate, and the radiotherapist calculated the positioning setup errors. RESULTS: Psychological interventions worked successfully with 45(85%) children behaved cooperative (without showing any resistances by crying and screaming) at first radiotherapy and keeping still until the session was completed. For the aged 3-year-old group, 64%(7/11) children was successfully behaved cooperative; 4-year-old children in the success rate of 77%(10/13); 5 and 6-year-old children in the success rate of 100%(16/16);7-year-old children in the success rate of 80%(4/5). above 8-year-old children in the success rate of 100%. For those failed cases, 1 child used anesthesia during the whole radiotherapy sessions; 6 children were take sedatives from the first to third session of radiotherapy. CONCLUSION: There is a notable efficiency of psychological interventions for children diagnosed with CNS tumor across the radiotherapy trajectory. Children, parents and clinical decisions preferred psychological intervention methods. Further research is required to strengthen the evidence base for psychological interventions in minimizing the use of anesthesia

QOLP-19. EFFECT OF INTENSIVE NUTRITION INTERVENTION ON PEDIATRIC PATIENTS WITH CENTRAL NERVOUS SYSTEM TUMORS WITH CONCURRENT RADIOCHEMOTHERAPY

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OBJECTIVE: Pediatric patients with central nervous system (CNS) tumors undergoing chemoradiotherapy are at high risk of malnutrition. The goal of this study was to investigate the effects of intensive nutrition intervention on the pediatric patients with CNS tumors receiving concurrent chemoradiotherapy. METHODS: We analysed retrospectively the clinical outcomes of 28 CNS tumor patients who were received early nutritional intervention (nutrition intervention group, NG) before they were treated with chemoradiotherapy. The outcomes of these patients were compared to that of 39 patients who received chemoradiotherapy without any early nutritional intervention (control group, CG). RESULTS: There are no significant differences between two groups in hemoglobin, platelet, prealbumin, globulin, and albumin before they were treated with chemoradiotherapy. However, the white cell count in control group was larger than that in NG (6.07 ± 1.35 vs. 8.87 ± 5.10p<0.05) (109/L). There are no significant differences between two groups in prealbumin, globulin and albumin after they were treated with chemoradiotherapy. The body mass percentage (5.08 ± 10.67 % vs. -0.49 ± 5.41%), white cell count (2.86 ± 1.00 vs. 2.31 ± 0.76) (109/L), hemoglobin count (120.20 ± 39.701 vs. 100.62 ± 14.44) (109/L) and platelet count (141.36 ± 47.95 vs. 101.11 ± 27.00 (109/L) in NG were higher than that in CG after they were treated with chemoradiotherapy. And the radiotherapy interruption time0.35 ± 1.20 vs. 2.07 ± 2.98 daysin NG were shorter than that in CG after they were treated with chemoradiotherapy. CONCLUSION: Early nutrition intervention can effectively improve the nutritional status of pediatric patients when they were treated with chemoradiotherapy. These results suggested that nutritional intervention must be initiated before chemoradiotherapy.

QOLP-20. QUALITY OF LIFE IN THE PHASE III CeTeG/NOA-09 TRIAL RANDOMIZING CCNU/TEMOZOLOMIDE (TMZ) COMBINATION THERAPY VS. STANDARD TMZ THERAPY FOR NEWLY DIAGNOSED MGMT-METHYLATED GLIOBLASTOMA

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The CeTeG/NOA-09 trial demonstrated a significant survival benefit of radiotherapy with CCNU/temozolomide (TMZ) combination therapy

compared to standard TMZ therapy as first-line treatment of O6-methylguanine-DNA methyltransferase (MGMT)-promotor hypermethylated glioblastoma. Quality of life (QoL) assessment was a secondary objective to investigate if CCNU/TMZ combination chemotherapy has a detrimental effect on patient's QoL. PATIENTS AND METHODS: Patients (n=141) received standard radiotherapy and were randomized (1:1) for CCNU/TMZ or standard TMZ. The modified intention-to-treat population consisting of 129 patients was analyzed. At least every three months, Karnofsky performance score (KPS) was determined and QoL was measured using the EORTC-QLQ C30 and BN20 questionnaires. A longitudinal mixed-model analysis was used to evaluate differences between treatment arms in the course of KPS and QoL over time. Time to first deterioration was analyzed using a Cox regression model. RESULTS: Over a period of four years, longitudinal mixed-model analysis detected no significant impairment of QoL or KPS in the CCNU/TMZ arm as compared to the TMZ arm. Time to deterioration was prolonged in one QoL domain, motor dysfunction, for patients in the experimental arm. CONCLUSION: Intensified alkylating chemotherapy with CCNU/TMZ for patients with MGMT promotor-methylated glioblastoma did not lead to a reduction of QoL or KPS compared to TMZ standard therapy.

QOLP-21. THE RELATIONSHIP BETWEEN CAREGIVING BURDEN AND ANXIETY SYMPTOMS IN CAREGIVERS OF PATIENTS WITH MALIGNANT GLIOMAS

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BACKGROUND: Patients with malignant gliomas have a poor prognosis and high symptom burden. Caregivers of these patients face significant challenges due to the impact of the cancer on their loved ones neurological and mental faculties. We have previously shown that rates of clinically significant anxiety symptoms in caregivers of this population are high (50%). We sought to evaluate whether caregiving burden was associated with anxiety symptoms in caregivers of patients with malignant gliomas. METHODS: We conducted a prospective study in patients with newly diagnosed malignant gliomas and their caregivers, collecting caregivers self-report data within 6 weeks of diagnosis. We assessed caregivers anxiety symptoms with the Hospital Anxiety and Depression Scale, with subscale scores >7 considered clinically significant. We evaluated caregiving burden using the Caregiver Reaction Assessment (CRA) subscales, with the following domains: impact on schedule, caregivers esteem, lack of family support, impact on health, and impact on finances. We used a linear regression model to identify associations between the CRA subscales and anxiety symptoms. RESULTS: Fifty percent (38/76) of caregivers experienced clinically significant anxiety symptoms. Caregiving burden was significantly associated with anxiety symptoms $F(5, 69)=11.525$, $p<0.001$, $R^2=0.455$. Impact on schedule ($B=2.273$, $p<0.001$), impact on health ($B=2.177$, $p=0.011$), and impact on finances ($B=1.374$, $p=0.002$) added significantly to the model. CONCLUSIONS: Half of caregivers of patients with malignant gliomas experience clinically significant anxiety symptoms within 6 weeks of the patients diagnosis. Caregiving burden accounted for a substantial proportion (45.5%) of the variance in anxiety symptoms, with impact on the caregivers schedule, health, and finances demonstrating significant relationships with anxiety. These results suggest that caregivers who report a greater caregiving burden that impacts their schedule, health, and finances are also at risk for heightened anxiety symptoms. Interventions to reduce caregiving burden and decrease caregiver anxiety are needed.

QOLP-22. THE INTERNATIONAL LOW GRADE GLIOMA REGISTRY: PATIENT-REPORTED QUALITY OF LIFE

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The overarching goal of the International Low Grade Glioma (LGG) Registry is to allow for focused study of LGG (defined as adult grade II astrocytoma, mixed glioma, or oligodendroglioma), including quality of life (QOL). To date, enrollment is completed for 234 patients from 21 states and nine countries (US, France, United Kingdom, Canada, Australia, Hong Kong, New Zealand, Belarus and Spain). Pilot data on QOL (MOS SF-36 scale, measured from 0–100 with higher scores suggesting better QOL) are available for 112 LGG patients for whom we have confirmed treatment via medical record review. Enrolled LGG patients were predominantly White (91.2%), female (56.3%), generally otherwise healthy with only 5% reporting a co-morbid condition) and had a mean age of 36.9 years. A wide range of symptoms were reported by patients: 23.4% reported being unable to drive, 32.4% reported trouble thinking, and 35.1% reported difficulty with getting words out. To date, fifty-two percent of patients have received radiation (XRT): patients not treated with XRT at diagnosis report significantly better physical functioning than do those who received XRT, with persons receiving XRT at time of diagnosis reporting the lowest scores ($p=0.003$). Interestingly, patients not treated with XRT reported lower emotional and mental health scores than did those receiving XRT ($p=0.02$). Interpretation of these findings is limited at this point given the small sample and possible selection bias; possibilities include that the no XRT group may be more depressed/anxious for unrelated reasons, or, the absence of treatment may leave them feeling uneasy. When compared to study subjects from our prior meningioma case/control study of QOL, these results suggest significant reduction in QOL for LGG patients and possible variation by XRT treatment and thus the need to better understand these differences.

QOLP-23. PALLIATIVE CARE AND END OF LIFE HEALTHCARE UTILIZATION IN PATIENTS WITH INCURABLE PRIMARY MALIGNANT BRAIN TUMORS

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BACKGROUND: Patients with primary malignant brain tumors (PMBT) experience high symptom burden and mortality. However, their healthcare utilization at the end of life, including palliative care utilization, has not been well studied. METHODS: We conducted a single-center, retrospective analysis of deceased patients diagnosed with PMBT between 1/1/12 and 8/31/15. We assessed healthcare utilization including hospitalizations, palliative care usage and hospice enrollment. RESULTS: We identified 200 patients with incurable PMBT within the above timeframe. The majority of patients (86.0%, 171/200) had a diagnosis of glioblastoma. Most patients were white (185/200, 93.0%), non-Hispanic (189/200, 95.5%), and male (117/200, 59.0%). Median survival was 13.8 months. When excluding hospitalizations for planned procedures or chemotherapy, the median number of hospitalizations was 2 (range 0–9). In the last 30 days of life, 61/200 patients (30.5%) were hospitalized. Notably, only 63/200 (31.5%) patients ever received a palliative care consultation. The majority of these patients (39/63, 61.9%) were seen by palliative care only as inpatients. Of those seen by palliative care, only 19/63 (30.2%) had their first contact in the outpatient setting. The median time from first palliative care consultation to death was 53 days (range 0–981) and from first inpatient consultation to death was 32 days (range 0–354). Most patients were enrolled in hospice prior to death (154/200, 77.0%), with a median length of stay in hospice of 25 days (range 0–405). The majority of patients (112/200, 56.0%) died at home, 6.5% (13/200) died in the hospital, while 29.5% (59/200) died in a skilled nursing or hospice facility. CONCLUSIONS: Despite the known poor prognosis in patients with PMBT, palliative care is used infrequently, late in the disease course and typically initiated only in the inpatient setting. As such, there is a need to develop and study interventions to ensure timely outpatient palliative care referral in this population.

QOLP-24. ATTITUDES TOWARD FERTILITY AND FERTILITY PRESERVATION IN WOMEN WITH GLIOMA

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BACKGROUND: No studies have examined the fertility priorities of women undergoing treatment for glioma. Glioma patients frequently undergo chemotherapy as part of their treatment, however it is unknown whether patients are aware of the possible effects of treatment on their fertility. Our objective was to assess the fertility priorities of glioma patients and ascertain whether female glioma patients are being effectively counseled on the effects of chemotherapy on fertility prior to beginning treatment. METHODS: The sample was composed of female patients from the Neuro-oncology clinic of the University of California, San Francisco. Participants completed a cross-sectional survey between October 2010 and December 2013 exploring their attitudes toward fertility and their experience with fertility counseling prior to chemotherapy initiation. RESULTS: Seventy-two women completed the survey. Analysis of the survey results showed that 30% of women receiving chemotherapy reported having a discussion regarding fertility preservation prior to beginning treatment. Of those who reported having this discussion, 80% were aware that chemotherapy could negatively affect their fertility. Many women reported that while fertility preservation was not important to them at the time of diagnosis, it was a priority for them at the time of survey completion. Although interest in having children tended

to decrease after cancer treatment, the majority of respondents reported desiring a child after treatment. CONCLUSIONS: The data obtained in this study suggest a lack of understanding of reproductive priorities which may be addressed with a more comprehensive fertility discussion prior to beginning treatment.

QOLP-25. QUALITY OF LIFE FOLLOWING RE-IRRADIATION FOR RECURRENT HIGH GRADE GLIOMA

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BACKGROUND: Re-irradiation has recently garnered interest as a treatment option for recurrent high grade glioma. Evidence supporting benefits in this population is evolving, but little is known about impact on quality of life (QOL). QOL patient reports were collected in a phase II hypofractionated re-irradiation study. METHODS: Patients with recurrent anaplastic glioma or glioblastoma were stratified into four groups based on histology and prior bevacizumab exposure. Treatment consisted of 45 Gy radiation delivered in 25 fractions with daily temozolomide 75 mg/m2 and bevacizumab 10 mg/kg every 2 weeks. This was followed by adjuvant daily temozolomide 50 mg/m2 for 6 weeks and bevacizumab 10 mg/kg every 2 weeks in an 8 week cycle for up to 6 cycles, or until progression or intolerance. FACT-Brain and FACIT-Fatigue were collected as secondary end-point data prior to treatment start, at end of the concurrent phase, and after each cycle. RESULTS: Of 54 patients treated, 42 (78%) had evaluable QOL data. Mean baseline FACT-Br and FACIT-Fatigue scores were within expected ranges for persons with brain tumors. Significant declines were noted in FACT-Br Total (p=0.4), FACT-Br TOI (p=.05) and FACIT-Fatigue (p<.01) at the end of concurrent treatment, but FACT-Br Total and FACT-Br TOI scores rebounded to baseline level by end of cycle 1 and were maintained at cycle 2 end. Fatigue scores improved after cycle 1 but did not return to baseline until after cycle 2. CONCLUSION: Re-irradiation with temozolomide and bevacizumab is associated with decline in QOL immediately after concurrent treatment but QOL improves over time. This QOL pattern is similar to that seen with initial radiation treatment and suggests that re-irradiation does not progressively adversely affect QOL for at least the first four months after treatment.

QOLP-26. PATIENT REPORTED OUTCOMES MEASUREMENT INFORMATION SYSTEM (PROMIS) SCREENING FOR ANXIETY & DEPRESSION IN CENTRAL NERVOUS SYSTEM(CNS) CANCER: LARGE COHORT REPORT FROM THE NEURO-ONCOLOGY BRANCH NATURAL HISTORY STUDY (NOB-NHS).

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BACKGROUND: Emotional distress, including anxiety and depression, is associated with significant morbidity. Studies in CNS tumor patients have been limited by small non-diverse samples. The NOB-NHS includes screening for depression and anxiety patients undergoing longitudinal care in the NOB. In a previously reported pilot study, anxiety(11%) and depression(15%), and association with performance status and use of psychotropic medications were reported. This report attempts to validate these findings in a larger more diverse cohort. METHODS: All patients(n= 269) enrolled in the NOB-NHS were screened for depression and anxiety using PROMIS measures. Descriptive statistics and standardized classification of moderate-severe depression and anxiety are used to describe the sample characteristics. Emotional Distress was defined as PROMIS T scores-> 60 on depression, anxiety, or both scales. Chi-square and Fishers exact tests were used to identify associations. Significance level was set at $p < 0.05$. RESULTS: The sample was primarily white(82%), males(61%), 54 months(0–398) from diagnosis, with a median age of 49(21–81), good performance status(KPS 90, 75%) and a high-grade neoplasm(63%) with glioblastoma(30%) the most common diagnosis. Significant emotional distress was present in 19% of the sample. Poor performance status(28%) and use of psychotropic medications(32%) were both significantly associated with emotional distress($X^2(1)= 4.8$, $p< 0.03$ and $X^2(1)= 7.4$,$p< 0.01$), respectively. Past recurrence(25%), progression/pseudo-progression on MRI(26%), female gender(23%), corticosteroid use(28%) and non-glioblastoma(21%) had higher incidence of distress, although not significant. Tumor location, age and disease status was not associated with emotional distress in this sample. CONCLUSION: This is one of the largest reported cohorts using screening measures for emotional distress in this population and confirms earlier findings of association with functional status and psychotropic medications. Future studies will investigate longitudinal trends, diagnostic referrals, and emotional distress phenotypes, including genomic predisposition, to improve individual patient/population care.

QOLP-27. USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE IN GLIOMA PATIENTS

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INTRODUCTION: Beside conventional tumor-specific therapy, glioma patients, as any cancer patients, may seek additional support of complementary and alternative medicine (CAM) treatment during the course of disease. CAM represents a heterogeneous field of agents and treatments, and the use and type may vary from country to country. Despite the existing demand from patients, the use of CAM is neither, by default, incorporated in clinical oncological routine, nor well assessed and documented to date, and little is known about type and motivation for CAM use in specific patient groups. MATERIAL AND METHODS: We conducted a multi-center cross-sectional survey analysis of CAM use in patients suffering from gliomas WHO grades II to IV, treated at specialized Neuro-Oncology Centers in Switzerland in the years 2012–2015. A questionnaire, comprising multiple choice questions as well as open response questions, was handed out to patients on the occasion of an appointment. RESULTS: A total of 208 patients returned the survey, of which 101 patients (49 %) reported the use of CAM, past or present. Of these, 60 were male and 41 female. The main reported motivation for CAM use was the desire to contribute actively to the cancer treatment. CAM use was associated with younger age and was distributed amongst all WHO grades. Usually, CAM use was not supervised by a health care professional, and the costs were not necessarily reimbursed by the insurance company. CONCLUSIONS: In this Swiss multi-center survey analysis, half of the patients harboring a glioma reported CAM use during the course of the disease. Physicians should be aware of this demand and explore CAM use in their patients, to allow for better counseling and avoid potential interactions of CAM with the tumor-specific therapy. Funded by the Swiss Brain Tumor Foundation

QOLP-28. FEAR OF DYING IN ADULT PRIMARY BRAIN TUMOR PATIENTS: AGE AND GENDER EFFECTS

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BACKGROUND: Given the lack of curative treatment options, brain tumor patients may have increased vulnerability to death anxiety, yet the literature to date remains sparse. Two consistent findings associated with death anxiety are age and gender effects, with younger adult women reporting higher rates. Furthermore, death anxiety is often underreported and therefore goes untreated, which can negatively impact quality of life and functioning. The purpose of the present study was to address these gaps in the literature by preliminarily investigating prevalence of and associations with death anxiety in brain tumor patients. METHODS: Eighty-six (51% male, age range 19–81 years) patients diagnosed with primary brain tumors (PBT; grades I-IV) participated in routine neuropsychological evaluations including emotional questionnaires. A single-item question from the Beck Anxiety Inventory (BAI) assessed fear of dying. Descriptive analyses, t-tests, and Pearson correlations were conducted to explore age and gender effects on patients' fear of death. RESULTS: Thirty percent (n=26) of patients reported fear of dying at the time of evaluation. Women endorsed higher fear severity than men (0.71 vs 0.33; $p = .048$). There was no relationship between fear severity and age. However, when examining the two effects together, there was a significant difference between men and women aged 30–39 years (0.00 vs. 1.17; $p = .035$). All descriptive statistics, comparisons, and relationships will be presented. CONCLUSIONS: Results suggest that a significant portion of PBT patients experience a fear of death, and this appears to be more severe in female compared to male patients. Age did not have an independent effect, but young women reported increased fear of death when compared to young men. These

results support literature on death anxiety in other populations. Findings could inform intervention development. Importantly, future research should focus on methods to improve early identification of death anxiety in PBT.

QOLP-29. SYMPTOM CLUSTERS IN NEWLY DIAGNOSED GLIOMA PATIENTS: WHICH CLUSTERS ARE ASSOCIATED WITH FUNCTIONING AND GLOBAL HEALTH STATUS?

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INTRODUCTION: Symptom management in glioma patients remains a challenge, as patients suffer from various concurrent occurring symptoms. This study aimed to identify symptom clusters and to examine the relation between these symptom clusters and functioning. METHODS: We prospectively included individual patient data from previously published international randomized controlled trials (RCTs) in glioma patients. Symptom prevalence and level of functioning were assessed with EORTC QLQ-C30 and QLQ-BN20 questionnaires. The magnitude of the associations between symptoms were examined with Spearman correlation coefficients and correlation matrices. Hierarchical cluster analyses (looking at euclidean distances) were performed to identify symptom clusters. Multivariable regression analyses were performed to analyze associations between the symptom clusters and functioning, adjusted for confounding variables. RESULTS: Data of 3595 newly diagnosed glioma patients who completed the questionnaires before randomization indicated that many patients (44%) suffered from 5–10 symptoms simultaneously. The most prevalent symptoms were fatigue, drowsiness and motor dysfunction, experienced by 86%, 59% and 55% of the patients respectively. Five symptom clusters were identified with hierarchical cluster analyses: motor cluster, fatigue cluster, headache cluster, gastrointestinal/seizures cluster and hair loss/itchy skin cluster. Having symptoms in the motor cluster had a clinically relevant and significant negative influence (≥10points difference) on the global health status/quality of life scale, and physical, role, cognitive and social functioning (Beta's ranged from -10.9 to -18.6, all $p<0.001$). Similarly, having symptoms in the fatigue cluster had a clinically relevant negative influence on the global health status/quality of life scale, and role and social functioning (Beta's ranged from -10.7 to -15.5, all $p<0.001$) CONCLUSIONS: Two symptom clusters, the fatigue and motor cluster, were frequently affected in glioma patients and demonstrated to negatively influence functioning and global quality of life. Implementation of interventions targeting fatigue and motor problems would therefore contribute to improved functioning and quality of life in glioma patients.

QOLP-30. SURVIVAL, LOCAL CONTROL, AND HEALTH RELATED QUALITY OF LIFE IN OLIGOMETASTATIC AND POLYMETASTATIC SPINAL TUMORS: A MULTICENTER, INTERNATIONAL STUDY

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BACKGROUND: Oligometastatic disease (5 metastases) is considered an intermediate cancer state of limited metastatic capacity and improved prognosis. Hence, in recent years, treatment of oligometastatic spinal disease has trended towards more definitive, ablative therapies. Little is known about the actual prognosis of patients presenting with oligometastatic spinal disease. The focus of this study was to compare oligometastatic spine patients to those with polymetastatic disease (>5 metastases). METHODS: This is an international, multicenter analysis of prospectively collected data. Data collected included demographics, survival, local control, histology, number and location of spine metastases, epidural spine cord compression (ESCC), the Spinal Instability Neoplastic Score (SINS), systemic disease burden, and treatment details. Health-related quality of life (HrQOL) measures included; numeric rating scale (NRS) for pain, EuroQol-5D (EQ-5D-3L), short form 36 version 2 (SF-36v2) and the spine oncology study group outcomes questionnaire (SOSGOQ). RESULTS: A total of 393 patients were included, of which 215 presented with oligometastatic disease and 178 with polymetastatic disease. A significant survival advantage was found for patients presenting with oligometastatic disease compared to those with polymetastatic disease at time of initial treatment of spinal metastases. This survival advantage was noticeable in both operative and non-operative patients. Local control rates were higher in the oligometastatic group for the spinal level treated. Furthermore, both groups experienced significant improvement in multiple HrQOL measures at 6 months post-treatment with no differences in these outcome measures between the two groups. CONCLUSION: At time of treatment of spinal metastatic disease, oligometastatic disease offers a significant survival advantage compared to polymetastatic disease, regardless of treatment choice. Local control can be achieved in both groups. HrQOL measures improve in both groups post-treatment, thus demonstrating a palliative treatment benefit for all treated patients. Our results support the oligometastatic theory and the current trend of ablative treatment for oligometastatic spine disease.

RADIATION BIOLOGY AND DNA REPAIR

RDNA-01. microRNA DEGRADATION MEDIATED GENETIC HETEROGENEITY AS A NOVEL MECHANISM FOR TEMOZOLOMIDE RESISTANCE IN GLIOBLASTOMA

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Temozolomide (TMZ), the standard-of-care chemotherapy for glioblastoma, is a DNA alkylating drug. At cytotoxic doses, TMZ Induces high levels of alkylated DNA, including O6 methyl-guanine (O6MG), ultimately triggering cell death. In sub-lethal levels, O6MGs undergo non-Watson-Crick pairing, facilitating mutagenesis and promote acquired resistance. Whether the cytotoxic or mutagenic effects of TMZ predominate is largely determined by the DNA repair enzyme, Methyl-guanine-methyl transferase (MGMT). MGMT restores TMZ-alkylated DNA into its native form. To date, studies of MGMT have largely focused on its expression in large populations of cells. Variation of MGMT expression at a single cell level and pertinence of this variation to acquired TMZ resistance remains poorly understood. In single cells derived from the same patient-derived glioblastoma line, we found that the cell-to-cell variability in MGMT mRNA expression differed by as much as 10-fold. The distribution of single cell MGMT expression approximated that of a normal distribution. Upon treatment with TMZ, the mean expression of MGMT increased by two-fold. Additionally, TMZ treatment increased the standard deviation of the MGMT expression distribution by ten-fold, causing significant "widening" of the range the MGMT expression. Both of these effects were caused by degradation of miR-181d, an MGMT suppressing miRNA. TMZ treatment triggered the activation of ATM- and Rad3- related (ATR) kinase, which in turn acti-

vated Polyribonucleotide nucleotidyltransferase 1 (PNPT1), an RNAse that ultimately degradation of miR-181d. Our results suggest that RNA degradation enhanced stochastic variation in MGMT expression, allowing a wider range of MGMT expression from which the optimally fit clone can emerge. This novel form of TMZ resistance can be suppressed by over-expression of miR-181d, suggesting the potential of microRNA delivery as a therapeutic strategy. Accordingly, miR-181d delivery combined with TMZ treatment effectively cured intracranial murine models of patient derived glioblastoma xenografts (PDX) that expressed high levels of MGMT.

RDNA-02. TUMOR TREATING FIELDS DIFFERENTIALLY ALTER HOMOLOGOUS RECOMBINATION IN PATIENT DERIVED GLIOMA CELLS VERSUS ESTABLISHED LINES

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Within the heterogeneous tumor cell population there exists a subpopulation exhibiting stem-like properties known as glioma stem cells(GSCs) which are thought to be prime contributors in tumor resilience. Low-frequency alternating electric fields, tumor treating fields(TTFs), have recently emerged as a novel form of radiotherapy with FDA approval for the treatment of glioblastoma. We sought to determine the anti-proliferative effects of TTFs using a panel of 4 patient-derived GSCs and established lines, U87 and U373. Using the Novocure Inovitro system, cells were treated with 200kHz TTFs for 72hr and the cell number was determined using a Vi-Cell Cell Viability Analyzer. We found a statistically significant reduction in cell growth following TTF exposure for 4 of the 6 cell lines tested. The size of the anti-proliferative effect was cell line dependent and varied between 15 and 30%. We examined the effect of TTFs on the DNA damage response following irradiation utilizing immunofluorescent foci staining kinetics coupled with high-throughput automated imaging and analysis. Our initial results demonstrated a varied response among the tested cell lines. A 48hr TTF application increased the number of 53bp1 foci above background in U87 and two of the tested GSCs. TTF application for 24hr prior to irradiation was found to systematically reduce the number of induced Rad51 repair foci. However, an increased number of persistent Rad51 foci in the established lines was measured, indicative of reduced repair. In contrast, within the GSCs, the initial reduction in Rad51 foci was maintained throughout the 24hr repair time course. Our preliminary results have demonstrated the efficacy of TTFs to reduce cancerous cell proliferation *in vitro* while uncovering a differential DNA damage modulation response between established lines and GSCs. Future studies will focus on elucidating the mechanism of GSC TTF resistance with the goal of increasing treatment efficacy via multimodal combination therapy.

RDNA-03. TARGETING THE EPIGENETIC REMODELING FACTOR FACT TO RADIOSENSITIZE GLIOBLASTOMA CANCER STEM CELLS

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Radiotherapy remains standard of care for glioblastoma (GBM) but has limited efficacy due to the ability of cancer stem-like cells (CSCs) to drive tumor recurrence through their enhanced malignant properties (i.e., radioresistance, angiogenicity, and invasiveness). Our overall objective is to address an unmet clinical need by identifying strategies to overcome the inherent radioresistance of GBM CSCs and develop effective treatment modalities that will inclusively target this recurrence-driving subset of cells. We have previously shown that components of the cell cycle and DNA damage response (DDR) machinery are required for CSC malignancy. More recently, we reported that the facilitates chromatin transcription (FACT) complex, which serves to reorganize nucleosomes to facilitate RNA polymerase II (Pol II)-mediated transcription, is a key mediator of the CSC phenotype. FACT has also been reported to have a role in DNA replication and the DDR. We hence sought to evaluate the impact of FACT disruption using the small molecule inhibitor curaxin-137, with proven blood brain barrier penetration, on the ability of CSCs to tolerate irradiation induced DNA damage. We first combined curaxin-137 treatment with irradiation and followed the resolution of DNA damage by immunofluorescence for γH2A.X or 53BP1, markers of DNA damage. Results indicated that the combinatorial treatment induced a significantly greater amount of DNA damage that failed to be resolved in the CSCs. We next explored the impact of treatment on CSC viability. Results indicated that combination treatment with curaxin-137 did reduce clonogenic survival of CSCs. Ongoing studies aim at evaluating the *in vivo* efficacy of this treatment as a radiosentizing agent. The ability to identify paradigms that increase the radiosensitivity of CSCs will have immense therapeutic implications on reducing the propensity of CSCs to drive the malignant phenotype of GBM.

RDNA-04. POTENTIAL MECHANISM OF TEMOZOLOMIDE-MEDIATED RADIO-SENSITIZATION IN MGMT HYPERMETHYLATED GLIOBLASTOMA CELLS

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Temozolomide (TMZ) mediated radio-sensitization is preferentially observed in MGMT hypermethylated glioblastoma (GBM) and is modulated by unknown underlying mechanisms. To gain insight into the potential mechanism of TMZ-induced radio-sensitization, we examined the effect of TMZ pre-exposure on the processing of radiation (RT)-induced double strand breaks (DSBs) in an isogenic model, the U251-vector versus U251-MGMT cells derived by the lentiviral MGMT transduction. Cells were treated with RT (2Gy) alone or after 2 days of pre-exposure to low concentrations of TMZ (20 μM) and survival was evaluated by clonogenic assay and DSBs by western blotting and immunofluorescence based γH2AX and RPA foci. TMZ pre-exposure significantly sensitized RT in U251-vector but not in U251-MGMT cells. In contrast, no significant difference was observed between RT alone and the concurrent RT/TMZ treatment. The radio-sensitization mediated by TMZ pre-exposure in U251-vector cells was accompanied with delayed DSB processing as evidenced by prolonged phosphorylation of KAP1(p-KAP1) and H2AX (γH2AX). Since DNA-PKcs plays a critical role in repair of RT-induced DSBs, we evaluated whether TMZ pre-exposure impacts the phosphorylation of DNA-PKcs in context of RT sensitization. Interestingly, we observed that TMZ alone induced phosphorylation of DNA-PKcs in both U251-vector and U251-MGMT cells and this effect was paradoxically suppressed by RT in time related manner, but in contrast, RT alone significantly increased p-DNA-PKcs. Further experiments confirmed that TMZ induces p-DNA-PKcs *in vitro* and *in vivo* and this effect was pharmacologically suppressed by ATR and ATM inhibitors. Importantly, RT-induced DSBs were decorated with p-RPA only in cells pre-exposed to TMZ suggesting single stranded DNA perhaps due to DSB end-resection. Together these results suggest that TMZ-mediated radiosensitization is due to delayed repair of RT-induced DSBs and the ongoing studies are focused on establishing a link between TMZ-mediated radiosensitization and TMZ-induced phosphorylation of DNA-PKcs.

RDNA-05. SYNTHETIC SENSITIZATION OF MGMT-DEFICIENT TUMOR CELLS TO TEMOZOLOMIDE USING ATR INHIBITORS

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BACKGROUND: Glioblastoma (GBM) is the most common primary brain tumor in adults. The current standard of care consists of surgery followed by temozolomide (TMZ) and radiation therapy (RT). TMZ is an alkylating agent that methylates guanine bases at the O^6 position (O^6meG). O^6-meG-methyltransferase (MGMT) is a suicide enzyme that repairs TMZ-induced damage by removing these adducts. Patients with methylation of the MGMT promoter respond more readily to TMZ and survive approximately 9 months longer than patients with an unmethylated MGMT promoter. Despite this prognostic benefit, these patients still have a median survival of only 21.7 months. The purpose of this study is two-fold: 1) to better understand the DNA damage response pathways that are activated in MGMT-methylated glioma cells upon treatment with TMZ, and 2) to determine if inhibiting these activated pathways sensitizes the glioma cells to TMZ. RESULTS: We found that TMZ, unlike other alkylators tested, specifically induces growth delay, DNA damage, and G2-phase cell cycle arrest in MGMT-methylated cells, but not in isogenic MGMT-positive cells. TMZ also induces markers of replication stress in MGMT-methylated cells, specifically pChk1 S345 and pRPA32 S33. As these proteins are markers of ATR activation, we hypothesized that inhibition of ATR would further sensitize MGMT-methylated cells to TMZ. *In vitro*, ATR inhibitors increased sensitivity to TMZ by over 1000-fold in MGMT-methylated cells, but not in MGMT-expressing cells. CONCLUSION: Here, we report exquisite synergistic interactions between TMZ and inhibitors of a key DNA damage response protein, ATR, in MGMT-deficient cells. These data lay the foundation for future clinical trials testing this combination specifically in MGMT-methylated glioma. Since ATR inhibitors are radiosensitizers, it is possible that this combination of chemotherapeutics will further synergize with RT in the treatment of GBM. Future experiments will examine this potential three-way synergy.

RDNA-06. A NOVEL ROLE OF SGEF IN MEDIATING GBM CELL SURVIVAL BY MODULATING THE DNA DAMAGE REPAIR MECHANISM

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Glioblastoma (GBM) is among the most genetically heterogeneous, treatment resistant, and lethal of all human cancers. A significant hurdle to effective treatment of GBM is the aggressive invasion of tumor cells into surrounding healthy brain tissue that invariably leads to tumor recurrence, brain injury, and patient death. These invasive cells preclude complete surgical resection and exhibit marked resistance to chemotherapeutics. As invasion is a universal property of GBM, studies focused on the invasive cell population and on the development of therapies that target them are greatly needed in order to significantly improve the survival of GBM patients. We have previously showed that SGEF (ARHGEF26), a RhoG-specific guanine exchange factor is overexpressed in high-grade brain tumors and correlates with poor patient survival. Here we report that SGEF is critical for promoting GBM invasion and survival by modulation of the DNA repair mechanism. Upon TMZ treatment, SGEF accumulated in the nucleus and mediated BRCA1 binding to γH2AX, and knockdown of SGEF expression in GBM cells impaired the phosphorylation of BRCA1 and CHK1. In addition, GBM cells with stable knockdown of SGEF expression showed enhanced susceptibility to TMZ induced cell death. Re-expression of SGEF in these cells rescued BRCA1 phosphorylation and glioma cell resistance to TMZ. Thus, our data showed an important role for SGEF in mediating GBM cell survival.

RDNA-07. IDH1-MUTANT GLIOBLASTOMA (GBM) CELLS FROM A PATIENT POST-TUMOR TREATING FIELDS (TTFIELDS) THERAPY ARE SENSITIVE TO TTFIELDS IN VITRO

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BACKGROUND: Tumor treating fields (TTFields) are FDA-approved for treatment of newly-diagnosed and recurrent GBM. TTFields extend progression-free survival and overall survival. Despite this advancement in patient outcome, many patients undergoing TTFields treatment ultimately progress with tumor recurrence. In this study, cell lines were generated from resected GBMs from two patients with secondary isocitrate dehydrogenase 1 (IDH1)-mutant glioblastoma. One patient received TTFields monotherapy for 1 year prior to resection, and one patient was TTFields-naïve. Cells were treated *in vitro* with TTFields followed by assessment of proliferative activity and cytotoxicity. METHODS: Tumors were enzymatically and mechanically disrupted to generate single-cell suspensions for culturing. Cells grew in DMEM/F12 media with 10% fetal bovine serum and gentamicin. Prior to TTFields application, cells were plated on plastic coverslips (2×10^4cells/coverslip) and incubated overnight. Then, TTFields were applied at 200 kHz (field intensity ~1.6 V/cm) for 14 days. Proliferation and cytotoxicity were assessed with XTT assay (n=6/group) and lactate dehydrogenase (LDH) release assay (n=3/group), respectively. Groups were compared by one-tailed t-test. RESULTS: After 14 days, proliferation was reduced in TTFields-treated cells (mean± SD; 0.763 ± 0.075) compared to control cells (1.064 ± 0.014; $p<0.002$) in the cells from the TTFields-treated patient, with no significant difference between groups in the cells from the TTFields-naïve patient (treated 1.269 ± 0.064; control 1.184 ± 0.097). However, in both cell lines, *in vitro* TTFields increased LDH release. In cells from the TTFields naïve patient, LDH release increased (12.62 ± 0.96) compared to control (7.375 ± 0.98; $p<0.002$). In the TTFields-treated patient cells, LDH release was 3-fold greater than control (31.890 ± 16.7 vs. 9.001 ± 1.21; $p<0.04$). CONCLUSION: TTFields decreased proliferation and induced cytotoxicity in IDH1-mutant GBM cells *in vitro*. This is the first report of *in vitro* effects of TTFields on GBM cells with mutated IDH1.

RDNA-09. RADIATION DRIVES THE EVOLUTION OF ORTHOTOPIC XENOGRAFTS INITIATED FROM GLIOBLASTOMA STEM-LIKE CELLS

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The clonal diversity and evolutionary dynamics inherent to GBMs is considered a major obstacle to effective treatment development. While studies have focused on temozolomide, a role for radiotherapy as a driver of GBM evolution has not been investigated. This study seeks to better understand the impact of radiotherapy, an important component of most treatment regimens, on GBM evolution. We intracranially implanted NSC11 and NSC20, CD133+ glioma stem-like cell lines, into nude mice. Bioluminescence imaging on day 21 post-implant confirmed the presence of tumor prior to randomization into control and radiotherapy groups (3x5Gy). Brain tumor xenografts were collected out to morbidity to assess morphological and histological changes following irradiation and to extract DNA for viral integration site analysis (VISA). Survival analysis demonstrates a significant survival advantage for mice undergoing radiotherapy (+31 days, NSC11; +36 days, NSC20). Gross examination at morbidity revealed brains bearing irradiated tumors contained tumor tissue that is more distinct, softer and more adherent, and more likely to efface olfactory bulb(s) than control tumor brains. H&E and SOX2 staining support these findings of a more infiltrative growth pattern in control tumors compared to irradiated tumors. VISA for NSC11 and NSC20 allowed for the examination of relative clonal diversity. Unsupervised hierarchical cluster analysis demonstrated a reduction in clonal diversity when transitioning from in vitro culture to in vivo conditions. Further reduction in clonal diversity is apparent when comparing control and irradiated tumors. To investigate whether the brain environment was necessary for the radiation-induced reduction in clonal diversity, NSC11 cells were irradiated in vitro and collected for VISA. Clonal diversity remained relatively consistent regardless of treatment. Our results demonstrate that radiation, in the context of the brain microenvironment, drives GBM evolution resulting in tumor morphology/histology modifications and tumor cell subpopulation selection. This process may have implications for treatment of recurrent GBM.

RDNA-10. HISTOPATHOLOGICAL AND GENOMIC CHARACTERIZATION OF GLIOBLASTOMA (GBM) RESECTED AFTER TUMOR TREATING FIELDS (TTFIELDS) THERAPY

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BACKGROUND: TTFields are FDA-approved for treatment of newly-diagnosed and recurrent glioblastoma. In this study, GBMs from one patient pre- and post-TTFields monotherapy were analyzed by immunohistochemistry (IHC) and array comparative genomic hybridization (aCGH) to examine potential TTFields-induced alterations. METHODS: Pre-TTFields (insular) and post-TTFields (anterior temporal and insular) GBMs were collected from a 38-yo male patient one year apart. Formalin-fixed tissues were studied by IHC utilizing the Vectastain ABC Elite kit (Vector Labs). Extracted DNA from GBMs was quantified and aCGH performed with Agilent SurePrint arrays (G3 ISCA CGH+SNP 180K) using a commercially-available, genetically-normal DNA standard. Bioinformatic analysis was performed with Agilent CytoGenomics Edition 2.5.8.1. RESULTS: Both pre- and post-TTFields tumors had mutated IDH1 and unmethylated MGMT promoter. Ki-67 stained 20–25% of tumor cells in the pre-treatment tumor, and 10–15% in the post-treatment insular tumor. CD133 staining was strong in the pre-treatment tumor with >50% cells stained, while CD133 staining in both post-treatment tumors was reduced in intensity and percent cells stained (<50%). MGMT intensity and number of stained cells was similar across all specimens. Overall, the aCGH analysis identified a small number of copy number gains in all three specimens, with far greater copy number losses. One notable difference between the pre- and post TTFields samples was a loss of the cytoband 13q12.12-q14.3 that was not found in the pre-treatment tumor ($\log_2$ fold change=-0.7496 to -0.8628; $p=1.41E-283$). This cytoband contains tumor suppressor genes retinoblastoma 1 (RB1) and breast and ovarian cancer susceptibility protein 2 (BRCA2). CONCLUSIONS: GBM specimens demonstrated reduced staining for Ki-67 and the stem cell marker CD133, and losses of RB1 and BRCA2 after TTFields therapy. Further studies are warranted as it is uncertain if these represent TTFields-induced changes, or typical GBM evolution.

RDNA-11. OVERCOMING RADIATION RESISTANCE IN MENINGIOMA: THE EMERGING ROLE OF CDK4/6 INHIBITOR

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Meningiomas are common intracranial brain tumors. Despite surgery and/or radiation therapy, recurrence rates occur in approximately 8–10 % of tumors. This may be due to the dysregulation of the cyclin D and cyclin-dependent kinase (CDK) pathway in malignant meningioma cells and radiation-resistant meningioma cells. The cyclin D-cyclin-dependent kinases 4 and 6 (CDK4/6)-retinoblastoma (Rb) pathway controls the cell cycle restriction point, and is commonly dysregulated in meningioma; making it a rational target for antimeningioma therapy. In this study, we interrogated the capacity of a CDK4/6 inhibitor, palbociclib, to activate RB function against Rb+ meningiomas cells in vitro, ex vivo and in vivo xenograft model. Meningioma tumors in SCID mice treated daily with intraperitoneal injections of palbociclib for 14 days dose dependly with X ray irradiation. Treatment effects were examined by immunoblot, cell viability, apoptosis, and cell cycle progression. Radiation resistance BENMEN1 cells were developed after exposure to repeated 320 kV X ray irradiation. Since CDK4 and CDK6 are proteins that are major part of a cell cycle regulatory pathway in meningioma that also includes p16, cyclin D, and the retinoblastoma (Rb)

protein, we analyzed those parameters. Our both *in vitro* and in vivo data clearly demonstrate that pallbociclib (CDK 4/6 inhibitor) treatment reduced proliferation and has additional effects on cell cycling, including induction of an RB-associated G(1) arrest against Rb+ malignant meningioma cells and radiation-resistant meningioma cells. We did not see any significant effect of palbociclib on Rb – meningima cells. Our results also demonstrated that palbociclib treatment inhibits CDK4 and CDK6 expression and also decreases E2F target gene expression (CCNA2 and CCNE2). All together, our results provide strong evidence that palbociclib can be applied to Rb+ meningiomas as a therapeutic agent.

RDNA-12. ATR INHIBITOR VE-822 IS A NOVEL RADIO- SENSITIZER FOR GLIOMA

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Glioblastomas (GBM) are lethal brain tumors for which surgical resection, treatment with ionizing radiation (IR) and concurrent administration of Temozolomide (TMZ) is the mainstay of therapy. These tumors are extremely radioresistant, and resistance to radiation is one of the primary causes of therapy failure. Therefore, there is an urgent need to overcome radioresistance in GBM in order to develop effective therapies for treatment. IR induces double strand breaks (DSBs) in the DNA, and these are extremely deleterious lesions that can be repaired either by error-prone non-homologous end joining (NHEJ) or the error-free homologous recombination (HR) pathway. Our laboratory has shown that the 5'-3' exonuclease EXO1 is crucial for DNA end resection and accurate repair pathway choice. We have previously shown that EXO1 is phosphorylated and activated by CDKs 1 and 2 in the S and G2 phases of the cell cycle which results in promotion of error-free HR as well as activation of the ATR kinase. Once activated, ATR phosphorylates EXO1 and targets it for degradation thereby preventing hyper-resection and genomic instability. Therefore, we hypothesized that ATR inhibitors could augment radiotherapy by stabilizing EXO1. Indeed, we find that that a specific ATR inhibitor - VE-822 - blocks IR-induced EXO1 degradation in a panel of GBM cell lines resulting in hyper-resection and attenuation of HR. Hence, treatment with VE-822 sensitizes GBM lines to IR in vitro. In vivo, we show that the drug can cross the blood-brain barrier and, in conjunction with IR, can block DNA repair in intracranial tumors, attenuate tumor growth and consequently extend survival of tumor-bearing mice. Our results indicate that negative regulation of EXO1 by ATR is critical for optimal DSB repair, and that this connection can be subverted by ATR inhibitors in order to improve GBM radiotherapy in the clinic.

RDNA-13. VALIDATION OF BEHAVIORAL ANALYSIS ACROSS AGE IN A MOUSE MODEL FOR FUTURE INVESTIGATION OF RADIATION-INDUCED HYPERSOMNOLENCE (RIH) IN PRIMARY BRAIN TUMOR (PBT) PATIENTS

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BACKGROUND: Cranial radiation is part of standard PBT treatment with patient age recognized to impact response and toxicity. RIH is one of the most common toxicities. We have reported an association between circadian gene variants and RIH susceptibility. No rodent model for RIH has been validated. The purpose of this study is to evaluate the impact of age on activity and circadian behavior to inform our novel mouse model of RIH. METHODS: Ethovision XT Software and Phenotyper cages wereused to continually video animal behavior and generate general activity data. Male young (6wk, n=3) or old (18mo, n=3) C57BL/6 mice were individually housed. Mice were monitored for 10 days under 12:12hr light:dark conditions. The data was examined for activity and circadian parameters. RESULTS: Older mice had lower total activity levels (-36%) than younger controls, with greatly decreased levels during the active period (-49%) but increased levels during the sleep period (49%). Actograms of general activity binned in 10 min intervals demonstrated a dramatic impact of age on circadian rhythms. Older mice displayed a dampened amplitude of activity rhythms (-36%; t= 4.1, $p<0.01$) and higher phase angle of entrainment (40.3 ± 25.8min) compared to younger mice (-1.11 ± 0min). Earlier activity onset in older animals increased the total activity period (alpha) by 7% (t=2.9, $p<0.05$). Both general activity and circadian data collected with Ethovision XT provided clear validation of the technique. CONCLUSIONS: Activity, sleep and circadian rhythms are impacted by irradiation in patients. A good behavioral model to observe these changes has not yet been developed in rodents. Here we demonstrate a reliable system to collect/analyze both activity and circadian parameters. Our study demonstrates a clear difference in all parameters at age extremes for control animals, a factor that warrants inclusion in our RIH model designed to recapitulate the human experience.

RDNA-14. IDENTIFICATION OF NOVEL RADIOSENSITIZERS IN GLIOBLASTOMA CANCER STEM-LIKE CELLS

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Glioblastoma (GBM) is the most lethal primary brain malignancy characterized by rampant genomic instability, angiogenesis and intra- as well as inter-tumoral heterogeneity, albeit leading to therapeutic resistance. Substantial evidence has identified glioblastoma stem cells (GSCs), to be the source of therapeutic resistance and tumor recurrence. In comparison to their differentiated counterparts, GSCs exhibit increased DNA damage response (DDR) signaling, which at least in part contributes to their radioresistant phenotype. To identify novel factors that drive the DNA Damage Responses in GSCs and so contribute to their radio-resistance, we performed a medium-throughput siRNA microscopy screen using a library targeting genes involved in chromatin remodelling. The primary screen identified a number of novel players sensitizing GSC population to ionizing radiation. We believe that our findings will contribute to better understanding of the mechanisms employed by GSCs to evade radiotherapy.

RDNA-16. THE ROLE OF RAD52 IN GENOMIC INSTABILITY AND THERAPEUTIC RESISTANCE OF MALIGNANT GLIOMAS

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Glioblastoma (GBM) is the deadliest and most common primary brain tumor with median survival rates of less than two years. GBM is defined by hallmarks such as uncontrolled cellular proliferation, diffuse infiltration, propensity for necrosis, robust angiogenesis, resistance to apoptosis and rampant genomic instability. Inter- and intra-tumoral heterogeneity, cellular plasticity and de-regulated signaling pathways are plausible causes of resistance to existent therapies in GBM. As current therapies offer only limited survival benefits, the identification and validation of new approaches in glioblastoma management is of highest importance. RAD52 is one of the key homologous recombination (HR) proteins. Rad52 binds to single strand DNA (ssDNA) and so plays a crucial role in most HR events as well as restart of collapsed replication forks in response to oncogene-induced replication stress. Our recent findings show significantly higher Rad52 mRNA levels in malignant gliomas compared to normal brain, where its levels negatively correlate with GBM patient survival. Experimental data show that the inhibition of RAD52 by 6-Hydroxy-DL-Dopa (6OH-Dopa) impairs GBM cell survival due to increase in replication-transcription collisions leading to accumulation of DNA damage. These preliminary findings support for first time a novel role of RAD52 in glioblastoma. We believe that Rad52 targeting may hold promise in future therapeutic intervention in GBM

RDNA-17. POWER DENSITY LOSS CAN BE USED TO DEFINED TUMOR TREATING FIELDS DOSE

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Tumor Treating Fields (TTFields) are alternating electric at 200 kHz approved for the treatment of Glioblastoma Multiforme. Historically, TTFields dose has been quantified using the magnitude of the electric field, indicative of the force that the field applies on charged objects. However, when considering TTFields dose, it is important to consider the amount of energy transferred from the electric fields to the tissue. This energy quantifies the extent to which the field alters the state of the objects on which it operates. Since power loss density quantifies the energy transferred by an electric field to tissue, we analyzed the power loss density distributions when TTFields is delivered to the brain. The analysis was performed by numerically simulating delivery of TTFields to computational models of glioblastoma patient. The simulations showed that TTFields magnitude tends to be higher in regions of low conductivity and tends to decrease in regions of high conductivity such as the ventricles and resection cavities. On the other hand, power loss density tends to increase in regions of higher conductivity. Within the highly conductive ventricles and resection cavity, it can take on values comparable to those observed in other tissue types. The total power loss of TTFields within the simulated cases was between 20–40 Watts, which is equivalent to 412–825 Kcalories per day, on-par with the resting metabolic rate of the brain (about 20% of the body's resting metabolic rate). This analysis shows that power loss density is a viable measure for quantifying TTFields dose. The observation that the power delivered by TTFields to cells

is comparable to the metabolic rate of the cells could lead to new insights into the mechanism of action of TTFields.

RDNA-18. TP5, A PEPTIDE INHIBITOR OF ABERRANT AND HYPERACTIVE CDK5/p25: A NOVEL THERAPEUTIC APPROACH AGAINST GLIOBLASTOMA

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BACKGROUND: Increasing evidence suggests a role of increased expression of CDK5 in cancer progression. Here, we examined the efficacy of selective inhibition of CDK5 in glioblastoma, the most frequent and aggressive primary brain tumor in adults. TP5 is a small peptide developed by National Institutes of Health which selectively inhibits aberrant and hyperactive CDK5/p25 while preserving the physiological CDK5/p35 functions. This peptide was modified to facilitate passage through blood brain barrier. METHODS & RESULTS: We demonstrated that TP5 decreased the activity but not the expression of CDK5 and p35. TP5 alone, but not the scrambled peptide, impacted cell viability and colony formation of glioblastoma cell lines and increased early and late apoptosis. Analyzing the pH2A.X expression by Immunofluorescence and Western Blot, we observed that TP5 increased the DNA damage in a dose dependent manner. TP5 impaired the DNA repair by reducing the G2 phase and decreased the phosphorylation of ATM. Whereas CDK5 activity is increased by DNA-damaging agents such as temozolomide (TMZ) and irradiation (IR), we observed that the addition of TP5 to either TMZ or IR was synergistic on colony formation due to an accumulation of DNA damages. Concomitant use of TP5 and either TMZ or IR increased the pH2A.X foci number and expression, inhibited the G2 arrest induced by these therapies and the phosphorylation of ATM. TP5 alone (300 mg/kg every 2 days) decreased the tumor volume of orthotopic glioblastoma mouse-model in a safety and dose-escalation experiment (4 mice per group: control, scrambled peptide, 100mg/kg, 300mg/kg). The treatment was well tolerated as the mouse weights were stable during the treatment and no hematologic, renal, pancreatic or liver function toxicities were observed (blood test). CONCLUSIONS: TP5 is a promising therapy for glioblastoma patients alone or in association with temozolomide and radiotherapy targeting a unique aspect of tumor biology.

RADIATION THERAPY

RTHP-01. DOES THE EFFICACY OF SALVAGE RE-IRRADIATION IN RECURRENT MEDULLOBLASTOMA DEPEND UPON RISK-STRATIFICATION, SITE OF RELAPSE, AND MOLECULAR SUBGROUP?

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PURPOSE: To report outcomes of salvage re-irradiation in the multimodality management of recurrent medulloblastoma. METHODS: Medical records of all patients with recurrent medulloblastoma treated with curative-intent re-irradiation at a single institution were analyzed. RESULTS: A total of 24 patients (median age of 14 years) were included. Median time to recurrence was 38 months (range 15–99 months). Of 13 patients with SHH subgroup medulloblastoma, 11 (85%) developed local recurrence while 5 of 7 (71%) group 4 patients developed focal recurrence outside the tumor bed. Neuraxial dissemination was seen in 2 patients each of SHH and group 4, while the lone patient with WNT-pathway experienced leptomeningeal dissemination. Molecular subgrouping was not known in 3 patients. Re-irradiation was focal in 58%, extended-field in 17%, and craniospinal re-irradiation in 25% patients. Median interval from primary course of irradiation was 49 months (range 24–98 months) and median cumulative biologically effective dose (BED) was 117 Gy (range 78–132 Gy). All patients received platinum-based salvage chemotherapy either before or after re-irradiation. At a median follow-up of 16.5 months, the 2-year post-re-irradiation progression-free survival and overall survival was 45% and 61% respectively. Patients with average-risk medulloblastoma at initial diagnosis fared better than high-risk disease, while site of relapse, and molecular subgrouping did not impact upon outcomes after re-irradiation. Two of 16 failures were in-field of re-irradiation indicating excellent local control. One patient developed symptomatic radiation necrosis. CONCLUSION: Re-irradiation provides excellent local control and encouraging survival outcomes with acceptable toxicity in carefully selected patients with recurrent medulloblastoma.

RTHP-02. IMPACT OF 18F-DOPA PET ON RADIOTHERAPY TARGET VOLUMES FOR NEWLY DIAGNOSED MGMT UNMETHYLATED GLIOBLASTOMA PATIENTS; PRELIMINARY RESULTS OF A PHASE II DOSE-ESCALATION TRIAL

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BACKGROUND: Multiple studies have demonstrated that amino acid PET tracers identify aggressive disease beyond what is contrast enhancing on T1-weighted conventional MR (T1CE). In this study, we explored discrepancies between 18F-DOPA PET and conventional MR for defining target volumes in newly diagnosed glioblastoma (GBM) radiotherapy patients. METHODS: In an ongoing NCI-funded trial (NCT:R01CA178200), 18F-DOPA PET was acquired prior to chemoradiation for newly diagnosed GBM patients and incorporated prospectively into the target volumes, modifying low and high dose target volumes, and guiding dose escalation to the most aggressive disease. Based on the results of our pilot biopsy-validation study, two 18F-DOPA PET volumes were defined, one with a T/N threshold >2.0 (PET_high) depicting the most aggressive disease and another with a T/N threshold default of ≥1.5 (PET_low) with modifications if needed by a Nuclear Medicine physician. Both PET volumes were compared with the T1CE+cavity MR volumes for 30 MGMT unmethylated newly diagnosed GBM patients. RESULTS: The PET_low volume extended beyond T2-FLAIR signal abnormality for all patients, and extended beyond standard CTV expansion in 60% of patients. Sixty-seven per cent of patients had at least 20% of the PET_high uptake outside the T1CE volume, and would not have been covered by standard CTV expansion in 8% of patients. The average (range) Dice Similarity Coefficient comparing T1CE with PET_high was 0.3(0.0–0.7). The average (range) Haursdorff maximum distance (cm) between PET uptake and T1CE was found to be 2.4(0.9–7.1). CONCLUSIONS: 18F-DOPA-PET identified regions of biologically active disease outside the T1CE in 67% of patients with significant discordance in volumes. Evaluation of the impact prospective 18F-DOPA PET guidance for dose escalated radiation therapy is under investigation in the ongoing trial.

RTHP-03. TIMING OF RADIATION THERAPY AFTER SURGICAL RESECTION OF INTRACRANIAL NON-SMALL CELL LUNG CANCER METASTASES: A RETROSPECTIVE ANALYSIS IN 28 PATIENTS

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BACKGROUND: The current treatment of brain metastases from the lung involves surgical resection of symptomatic lesions followed by adjuvant radiation therapy (ART) with no evidence-based guideline describing the ideal time from surgery to ART in this patient population. Our objective was to evaluate optimal post-operative timing of adjuvant RT with respect to survival outcomes. METHODS: We retrospectively identified 28 patients with pathologically confirmed primary non-small cell lung cancer and intracranial metastases who underwent a single craniotomy for resection with adjuvant RT between 2001 and 2016. Patient demographics, treatment details, and clinical outcomes were noted. RESULTS: Median surgery-to-radiation interval (SRI) was 31 days. We categorized patients into expedited RT (defined as SRI < 6 weeks, n=20) or delayed RT (SRI ³ 6 weeks, n=8). Patients receiving Delayed RT did not differ from patients receiving Expedited RT in demographics, pre-operative metastatic burden, post-operative functional status, or adjuvant RT modality. Fifteen patients (54%) were living at last follow-up (median follow-up 8.2 months, mean 20.3 months). Across all patients, expected overall survival (OS) was 21.4 post-operative months and progression-free survival (PFS) was 13.2 months. OS was significantly shorter after Delayed RT compared to Expedited RT (7.0 vs. 28.3 months, $P = 0.011$). PFS was 15.0 months with Expedited RT and 3.2 months with Delayed RT ($P = 0.39$). CONCLUSION: Our results suggest that delayed adjuvant RT with SRI ³ 6 weeks is associated with a shorter expected survival of up to 21 post-operative months. However, our study is limited by its retrospective nature, limited sample size, and limited follow-up. Refined studies are needed to more conclusively delineate optimal timing of adjuvant RT after surgical resection of intracranial lung cancer metastases, ideally involving well-powered randomized trials.

RTHP-04. TUMOR RECURRENCE OR RADIATION NECROSIS FOLLOWING CHEMORADIATION IN PATIENTS WITH GLIOBLASTOMA: DOES PATHOLOGY PREDICT OUTCOMES?

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Chemoradiation following surgical resection of glioblastoma has improved overall survival (OS). Unfortunately, all patients develop progression of the disease, typically characterized as worsening radiographic appearance. Radiological imaging is limited in differentiating tumor progression from radiation-induced necrosis; therefore, re-resection is often needed to remove the abnormal tissue. There is little and contradictory data assessing the relationship between pathological evaluation (active tumor vs. radiation necrosis) and clinical outcomes. We performed a large retrospective study to analyze the prognostic value and clinical significance of the pathology evaluation following re-resection in patients with presumed tumor progression. Clinical, radiographic and pathological information was compiled from 675 patients with GBM from the Memorial Hermann Health Care System between 2010 and 2017. Second surgery was performed in 121 (18%) patients (mean age=56, median KPS=80) with presumed progression following chemoradiation. According to the pathology report following second surgery patients were classified into 2 groups (1: recurrent tumor- # of patients = 91 and 2: radiation necrosis # of patients = 30). A Kaplan-Meier survival analysis was performed comparing the OS of each group. No statistically significant differences in OS from initial diagnosis (median OS: active tumor 22.6 mo, radiation necrosis 21.3 mo, p=0.4) and suspected recurrence (median OS: active tumor 12.7 mo, radiation necrosis 13.7 mo, p=0.3) were found between the groups. The current study suggests that histopathologic findings following chemoradiation cannot predict outcomes. These findings are important and should be considered to guide treatment strategies and clinical trial endpoints.

RTHP-05. NON-OPERATIVE TREATMENT OF NON-GERMINOMATOUS GERM CELL TUMORS OF THE PINEAL REGION

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OBJECTIVE: To retrospectively summarize the therapeutic effect of conventional radiotherapy combined with stereotactic radiosurgery (SRS) boosts in patients with NGGCTs of the pineal region, and explore an optimal non-operative treatment for improving patient's prognosis by increasing the radiotherapy dose of local focus dose. METHODS: NGGCTs of the pineal region treated in our hospital from 2008 to 2017 were retrospectively analyzed. Inclusive criteria: AFP (+) and/or HCG (+); the focus was located in the pineal region and cerebrospinal fluid implantation metastasis was excluded; postoperative image confirmed that the tumor was completely excised. The whole central nervous system radiotherapy combined with local conformal radiotherapy and SRS were performed in treatment group, and surgery as a salvage treatment was conducted when disease progression was confirmed. The patients in control group were treated with surgery combined with the whole central nervous system radiotherapy and local conformal radiotherapy. All patients received 6–8 courses of BEP regimen. RESULTS: A total of 26 patients were selected in this study, with a median age of 15 years (6–30 years). There were 17 cases in treatment group and 9 cases in control group. After being followed-up to 2018, 4 cases were lost and the median followed-up time was 35 months (8–100 months). For all 26 patients, the 3y-PFS and 3y-OS were 82.6% and 89.7%, respectively. During the follow-up period, disease progression was identified in 5 cases in treatment group (29.4%), 2 of which were treated with salvage surgery, with a 3y-PFS of 74.9% in treatment group. Two patients in treatment group were dead, with a 3y-OS of 85.6% in treatment group. No statistical differences were found in PFS and OS (P=0.119, 0.345). CONCLUSION: Conventional radiotherapy combined with local conformal radiotherapy and SRS boosts for NGGCTs of the pineal region is proved to be feasible.

RTHP-06. RANDOMIZED PROSPECTIVE TRIAL OF STEREOTACTIC RADIOSURGERY VERSUS CHEMOTHERAPY FOR RECURRENT MALIGNANT GLIOMA AFTER SECOND-LINE CHEMOTHERAPY

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PURPOSE: Outcomes for patients with recurrent malignant glioma are dismal. Fractionated radiosurgery (FSRS) has been shown to be feasible and safe when delivered in this setting, but prospective evidence is lacking. We conducted a single-institutional randomized trial on the use of FSRS versus chemotherapy for recurrent malignant glioma after second-line chemotherapy. METHODS: High-grade glioma patients with tumor progression after two previous treatment regimens were enrolled. They were randomized to FSRS with bevacizumab or bevacizumab with irinotecan, temozolomide, or carboplatin (discretion of the treating provider). FSRS was delivered as 32 Gy (8 Gy x 4 treatments within 2 weeks) to the gross target volume (gadolinium enhancing lesion and DWI abnormality), and 24 Gy (6 Gy x 4) to the clinical target volume (FLAIR abnormality). The primary endpoints were local tumor control (LC) at 2 months and progression-free survival (PFS). The study planned to accrue 78 patients total, but was closed early due to slow accrual. RESULTS: 34 patients were enrolled from February 2012 to December 2016. Twenty-seven patients had glioblastoma (WHO IV) and 7 had anaplastic glioma (WHO III). The median number of prior recurrences was 3. Patients on the FSRS arm had an improved PFS (5.3 vs 1.8 months, p< 0.001) and improved LC at 2 months (2/16 patients progressing at 2 months compared to 11/15 on chemotherapy alone) (p=0.001). The overall median survival was 6.4 months (7.1 months in the FSRS arm, 4.8 months in the chemotherapy arm, p=0.24). Five patients on the chemotherapy alone arm subsequently received FSRS at time of progression. CONCLUSION: Findings of our study suggest that FSRS in heavily pretreated patients with recurrent malignant glioma is feasible and improves LC and PFS when compared to treatment with next line chemotherapy alone.

RTHP-07. TRANSCRIPTION FACTOR NETWORKS OF OLIGODENDROGLIOMAS (IDH-MUTANT AND 1p/19q CODELETED) TREATED WITH ADJUVANT RADIOTHERAPY OR OBSERVATION INFORMS PROGNOSIS

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Multicentre sequencing efforts have allowed for the molecular characterization of low-grade gliomas (LGG)(The Cancer Genome Atlas (TCGA), 2015). We sought to analyze TCGA gene expression datasets on oligodendrogliomas patients treated with adjuvant radiation (RT) or those observed to discover prognostic markers and pathways. METHODS: mRNA expression and clinical information of patients with oligodendroglioma were taken from the TCGA "Brain Lower Grade Glioma" provisional dataset. Transcription factor network reconstruction and analysis were performed using the R packages "RTN" and "RTNsurvival". Elastic net regularization and survival modeling were performed using the "biospear", "plsRCox", "survival" packages. RESULTS: From our cohort of 137 patients, 65 received adjuvant RT and 72 were observed. In the cohort that received adjuvant RT, a transcription factor activity signature was generated that was associated with shorter progression-free survival (PFS) (HR = 2.3, p < 0.001). This increased risk was not seen in patients who were observed (HR = 0.8, p = 0.3). Within the observation cohort, transcription factors associated with the circadian clock pathway (ARNTL, ARNTL2, CLOCK) predicted for poorer PFS (HR = 1.6, p < 0.01). A transcription factor activity signature was generated that was associated with poor PFS (HR = 1.8, $p < 10^{-5}$) and OS (HR = 1.7, p < 0.002) only for those patients who were observed. Median OS in the observation cohort negative for the signature was not reached, but was 70 months for patients positive for the signature. CONCLUSIONS: We identified a transcription factor activity signature associated with poor prognosis in patients with IDH mutated and 1p19q codeleted oligodendroglioma treated with adjuvant radiotherapy. These patients would be potential candidates for treatment intensification. A second signature was generated for patients who were more likely to progress on observation. This potentially identifies a cohort who would benefit from upfront adjuvant radiotherapy.

RTHP-08. RE-EVALUATING THE SEQUENCING OF RADIOTHERAPY AND CHEMOTHERAPY IN PEDIATRIC MEDULLOBLASTOMA

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BACKGROUND: Standard treatment for medulloblastoma in children over age 3 includes resection and radiotherapy, followed by chemotherapy. Some studies have found disease control decrements if chemotherapy was delivered before radiotherapy. However, these studies recommended radiation interruptions for hematologic toxicity, which were more severe in children receiving chemotherapy prior to craniospinal radiotherapy. We report outcomes of pediatric medulloblastoma patients treated postoperatively with radiotherapy-first (RT1) or chemotherapy-first CT1). POPULATION/METHODS: 206 patients age 2–23 years with medulloblastoma were treated with proton radiotherapy from May 2000 to December 2016. We analyzed the effect of sequencing radiotherapy first (n=164) or chemotherapy first (n=42) after surgery on event-free (EFS) and overall survival (OS) controlling for known risk factors. RESULTS: Median follow-up was 5.8 years. Children who received CT1 sequencing were younger (p<0.0001), more likely to receive high-dose chemotherapy (p<0.001), have high/intermediate risk disease (p<0.0001), M1-M3 stage (p<0.001), and large-cell/anaplastic histology (p=0.04). M1-M3 stage (p=0.01), high/intermediate risk disease (p=0.03), and anaplastic/large-cell histology (p=0.04) were all predictive of worse EFS. Despite higher proportions of risk factors in the CT1 cohort, 5-year EFS and OS in the RT1 versus CT1 groups were no different at 89% versus 84% (p=0.39) and 90% versus 84% (p=0.33), and remained equivalent when controlling for M1-M3 stage and unfavorable histology (p=0.86). Radiation treatment duration was equivalent at a median of 43 days (RT1) and 42 days (CT1) (p=0.4). Children with anaplastic/large-cell histology had lower EFS only in the RT1 cohort (64% vs. 87%, p=0.01) and equivalent EFS in the CT1 cohort (73% vs. 78%, p=0.88), suggesting a possible benefit to high-dose chemotherapy before RT in children with unfavorable histology. CONCLUSIONS: Disease control and overall survival were equivalent in the radiotherapy-first and chemotherapy-first cohorts when radiation treatment times were minimized.

RTHP-09. DYSREGULATION OF Wnt SIGNALING PATHWAY CORRELATES WITH TREATMENT OUTCOME IN GLIOBLASTOMA MULTIFORME

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Glioblastoma multiforme (GBM) is the most common and most aggressive primary brain cancer with a median life expectancy of 13–15 months after surgery and chemo-radiation treatment. GBM is hallmarked by a poor response to radiation therapy (RT), which is generally attributed to upregulation of several DNA repair pathways. To identify pathways which predict poor chemo-radiation treatment outcome, we conducted a retrospective clinical-genetic study in which we evaluated the mRNA expression profiles of 24 GBM tumor samples, stratified on the basis of patient survival post chemo-radiation treatment. All patients were treated at our institution by surgical resection and intensity-modulated RT with concurrent and adjuvant temozolomide. Expression profiles were obtained utilizing a commercially available PanCancer Pathways Panel (NanoString Technologies, Inc.). Our data analysis shows a distinctly significant correlation between 5 members of the Wnt pathway and overall patient survival. A 2- to 9-fold higher expression of negative regulators of the Wnt pathway and a 2- to 3-fold lower expression of positive Wnt regulators was observed in tumor material of patients with a longer than median survival. We conclude that upregulation of the Wnt pathway correlates with poor chemo-radiation treatment outcome in GBM. *In vitro* experimentation by other groups has recently demonstrated a role for Wnt signaling in activation of Non-Homologous End-Joining mediated DNA double-strand break repair and the subsequent onset of radio-resistance. These preliminary findings support the hypothesis that the Wnt pathway may be used as a predictive marker for RT outcome in GBM and suggest new strategies to increase the radiation sensitivity of GBM.

RTHP-10. LESS IS MORE OR BIGGER IS BETTER? RADIATION TREATMENT VOLUME FOR GLIOBLASTOMA PATIENTS DOES NOT IMPACT SURVIVAL

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PURPOSE: To investigate if the extent of the planning target volume (PTV) for glioblastoma (GBM) patients receiving adjuvant radiation treatment (XRT) has correlation with survival. METHODS: We retrospectively examined patients with newly diagnosed GBM received adjuvant radiation at our institution from 2011 to 2016. Our institution follows the RTOG guidelines for GBM volumes. We included 87 patients with sufficient follow up. We examined the treatment plan documents to determine the PTV treated to 46 Gy (PTV46) and 60 Gy (PTV60). We measured overall survival (OS) as well as progression-free survival (PFS). We performed summary statistics on baseline patient characteristics, Kaplan-Meier analysis for outcomes with log-rank tests to compare PTV subgroups as well as Cox regression analysis for treatment volume. RESULTS: Of the 87 patients for analysis, 42 were female and 45 were male. Median age was 61 y/o, with median follow-up of 16.6 months (range 2.6 to 61.1 months). For PTV46 analysis, 1 patient was excluded due to receiving whole brain radiation to 40 Gy before receiving cone down to total 60 Gy. Median PTV60 was 297.9 cc (120.04 to 907.6 cc) while median PTV46 was 452 cc (120.04 to 907.6 cc). OS at 1 year was 67.8% and at 2 years was 32.2%, with median OS of 16.6 months. PFS at 6 months was 58.6% and at 1 year was 27.6%, with median time to progression of 6.8 months. On Cox regression analyses, neither PTV46 nor PTV 60 were statistically significant correlated with OS (p=0.11 and 0.68) and PFS (p=0.54 and 0.64). On log rank test, neither PTV46 nor PTV60 subgroups have any statistically significant survival differences, for PFS or OS. CONCLUSION: Extent of PTV does not appear to have an impact on recurrence or survival for GBM. Further validation with an independent dataset is warranted.

RTHP-11. REIRRADIATION OF RECURRENT HIGH GRADE GLIOMAS: OUTCOMES AND PROGNOSTIC FACTORS

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PURPOSE/OBJECTIVE(S): Identify prognostic factors for progression-free survival (PFS) and overall survival (OS) after reirradiation (re-RT) for recurrent high grade glioma (HGG). MATERIALS/ METHODS: Patients with HGG received re-RT from 2010 to present. PFS and OS prognostic variables were examined using Cox models. Receiver operative curve (ROC) analysis identified predictive thresholds for continuous variables. RESULTS: 58 patients received surgery and adjuvant radiation for HGG (51 grade IV, 7 grade III). The median time to first progression after initial radiation was 11 months. 36% received single fraction stereotactic re-RT (SRS) (median 18 Gy) and 64% received fractionated re-RT (median 35 Gy in 10 fractions). The median planning target volume (PTV) was 16.8 mL. The median biologically effective dose (BED10) of re-RT was 47 Gy (range 15–72). 50% received chemotherapy and 36% received bevacizumab concurrent to re-RT. Toxicity ≥ grade 3 was 7%. The median PFS after re-RT was 4.7 months and the median OS was 11 months. Lower PFS was significantly associated with shorter time to first progression, lower KPS, and lower re-RT dose. Lower OS was associated with shorter time to first progression, lower KPS, and larger PTV. Other factors were not significantly associated with PFS or OS. ROC analysis of time to first progression and re-RT dose showed best predictive thresholds at time > 12 months and BED10 > 42 Gy. CONCLUSIONS: Reirradiation was tolerated with infrequent high grade toxicity. PFS and OS after re-RT were both predicted by time to progression after initial radiation. Published prognostic scores have used total time from first to second radiation courses; however in our series the period from initial progression to re-RT did not add prognostic information. There was evidence for a dose threshold irrespective of radiotherapy technique. Use of chemotherapy and bevacizumab with re-RT were not associated with improved outcomes.

RTHP-12. COMPARATIVE ANALYSIS OF TUMOR TREATING FIELDS USING CONVENTIONAL VERSUS ALTERNATIVE ARRAY PLACEMENT FOR POSTERIOR FOSSA GLIOBLASTOMA

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BACKGROUND: There is an alternative transducer array placement configuration to treat infratentorial tumors using TTFields but coverage for posterior fossa glioblastoma is unknown. METHODS: Patient anatomy-based models were created by segmenting MRI images into tissue "masks". The physical properties and boundary conditions for physics modeling were set up within COMSOL Multiphysics. Electric field maps were compared for models using conventional array placement for supratentorial tumors versus alternative array placement for infratentorial tumors. Electric field–volume histograms (EVHs) and specific absorption rate–volume histograms (SARVHs) were constructed to evaluate volumetric differences between models. RESULTS: The alternative configuration consists of array placement at the vertex, the bi-occipital regions and the upper neck. Highest E_{AUC} was found at the epidural space surrounding the spinal cord and scalp for both types of configurations, whereas the lowest was located at the

tongue and orbits. Using the conventional configuration, the gross tumor volume (GTV) had an electric field area under the curve (E_{AUC}) of 40.5 V/m, volume covered with electric field intensity of 150 V/m (V_{E150}) of 0.01%, 95% electric field intensity ($E_{95\%}$) of 30.9 V/m, $E_{50\%}$ of 41.1 V/m, and $E_{20\%}$ of 46.6 V/m. The GTV also had a SAR_{AUC} of 4.0 W/kg, volume covered with SAR of 6 W/kg (V_{SAR6}) of 0%, $SAR_{95\%}$ of 0.6 W/kg, $SAR_{50\%}$ of 0.7 W/kg, and $SAR_{20\%}$ of 0.8 W/kg. The alternative configuration produced E_{AUC} of 52.3 V/m, V_{E150} of 3.6%, $E_{95\%}$ of 29.1 V/m, $E_{50\%}$ of 44.7 V/m, and $E_{20\%}$ of 58.1 V/m, as well as SAR_{AUC} of 0.9 W/kg, V_{SAR6} of 0.3%, $SAR_{95\%}$ of 0.6 W/kg, $SAR_{50\%}$ of 0.8 W/kg, and $SAR_{20\%}$ of 0.9 W/kg. CONCLUSIONS: The alternative array placement provides a higher coverage of electric field (+29%) to the posterior fossa glioblastoma when compared to the conventional configuration.

RTHP-13. TUMOR-TREATING FIELDS THERAPY IS COMPATIBLE WITH STANDARD CHEMORADIOTHERAPY FOR GLIOBLASTOMA

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PURPOSE: The phase III EF-14 trial demonstrated a significant overall survival benefit in 695 patients with glioblastoma who received adjuvant anti-mitotic tumor-treating fields (TTFields) therapy in addition to temozolomide (TMZ), following standard concurrent radiotherapy (RT) and TMZ. Preclinical studies suggest that there may be an added synergistic benefit to TTFields when used concurrently with RT. We therefore conducted dosimetric analyses to assess whether the presence and repositioning of TTFields scalp arrays interferes with RT delivery and to evaluate implications for treatment planning. METHODS: RT plans from ten consecutive glioblastoma patients at our institution were transferred to an anthropomorphic cranial phantom and re-optimized using standard tumor and normal tissue constraints. Optimized plans were then copied to CT scans of the phantom wearing the TTFields array in three discrete positions, to simulate routine replacement of the array in clinical use. Individual and average perturbations to RT dosimetry for each plan/array combination were then analyzed. RESULTS: The percent change in planning target volume (PTV) coverage for each of the 30 combinations ranged from -3.0% to 1.1%. When averaged over the three array positions, PTV coverage for each patient had standard deviation of less than 1.0%. Changes in PTV coverage were attributable to attenuation by the array and increased superficial dose owing to bolus effect. Percent increase in radiation dose to skin was 5.0% or less in 9 out of 10 plans. CONCLUSION: The dosimetric impact of the TTFields arrays for any individual position is small and is further mitigated by repositioning of the arrays as would occur during chemoradiation. These dosimetric changes were consistent across varied tumor location, laterality, and RT beam arrangement in our patient cohort. We are initiating a clinical trial to test safety and develop procedures for the use of TTFields during chemoradiotherapy for glioblastoma.

RTHP-14. TUMOR-TREATING FIELDS FOR GLIOBLASTOMA: NUMERICAL SIMULATION EXPLORES SUB-CELLULAR MECHANISMS

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INTRODUCTION: Tumor Treating Fields (TTFields) 100–500 kHz electric fields at ~1–4 V/cm exert an anti-mitotic effect on cancer cells. Our goal is to uncover TTFields mechanism by numerically modeling their effects on sub-cellular structures, notably microtubules (MTs). METHODS: We built a geometrically accurate finite element model in COMSOL Multiphysics (tm) of the MT and its micro-environment as a layered 27 nm-diameter cylinder: the inner lumen; 13 helical strands of alpha-beta tubulin dimers; C-termini; counter-ion layer; and an outer non-conductive Bjerrum layer. RESULTS: Modeling current density induced in each layer by TTFields for MTs varying in length from 1 10 μm showed that MTs act as electrical shunts conducting electric current within them. The resulting strongest current flows through the counter-ion layer surrounding the C-termini and energy density in this layer exceeds the level likely to disrupt the motor protein kinesin walk along the C-termini. The energy density is highest predicted at 1e-20 Joules when both the field and the MTs are aligned with the cell axis, in accord with in vitro experiments. A second mechanism predicted by our model is disruption of the foot of kinesin, released from its C-terminus contact by ATP (1e-19 Joules). The final phase of the walk is driven by thermal buffeting of the forward foot randomly positioning it near enough to the C-terminus for electrostatic forces to bind it. A stall force ~1e-19 - 1e-16 N from TTFields would prevent diffusion and disrupt the kinesin walk. CONCLUSION: Our modeling predicts that TTFields in cytosol induce electric currents along MTs that are strong enough to disrupt key cellular functions such as the kinesin walk and C-termini transitions, which are crucial for motor protein transport. Hence, TTFields disrupt the most delicate mechanisms involved in the carefully-orchestrated succession of steps in mitosis.

RTHP-16. RADIATION INDUCED SIGNAL CHANGES ON MAGNETIC RESONANCE IMAGING IN ADULT PATIENTS WITH BRAIN TUMORS TREATED WITH PENCIL BEAM SCANNING PROTON THERAPY

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OBJECTIVES: We report radiation induced signal changes (RIC) on MRI in adult patients with brain tumors treated with pencil beam scanning proton therapy (PBS-PRT). METHODS: All patients > 18 years receiving standard fractionation RT (4554 GyE) for a primary brain tumor with > 6 month follow-up were included. Post-RT MRIs were compared to the pre-RT scans. RIC was defined as new contrast enhancement (CET1W) and/or T2-FLAIR (T2W) changes MRI outside the GTV (CTCAE v4.0 grading was used). Monte Carlo, RBE (relative biologic effectiveness), and LET plan evaluation pre-RT were performed. Tumor, clinical and treatment factors were analyzed. RESULTS: Twenty-one patients were identified. RIC developed in 17/21 (81%: 2 CET1W, 15 T2W). All RIC appeared adjacent to surgical cavity and/or GTV in areas of increased RBE. 3 patients developed symptomatic RIC: All three patients had CET1W changes within tumor. Patient 1 received 50.4 GyE for a subtotally resected skull base meningioma. T2W changes developed at 3 months and CET1W changes in the tumor appeared at 7 months. Grade 2 symptoms (headache, unsteadiness, incontinence) developed by 14 months. Symptoms and imaging improved with dexamethasone taper by 22 months. Patient 2 received 54GyE for a subtotally resected grade II oligoastrocytoma. 4 months post-RT patient developed perilesional T2W. Headaches developed at 7 months and resolved following dexamethasone taper. Patient 3 received 54GyE for a progressive tectal plate glioma. At 2 months post-RT, CET1W changes within the tumor and T2W perilesional changes developed. Grade 3 diplopia developed a month later. The CET1W and T2W changes resolved at 13 months. No relationships between factors and RIC were identified. CONCLUSION: Asymptomatic RIC are common after RT, but symptoms are infrequent. Symptoms appear to be associated with post-PRT changes within tumor. A larger cohort including comparison to photon treatments and RBE will be evaluated in the future.

RTHP-17. PATTERNS OF RE-IRRADIATION FOR RECURRENT GLIOMAS AND VALIDATION OF A PROGNOSTIC SCORE

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Re-irradiation is a generally accepted method for salvage treatment in patients with recurrent glioma. However, no standard radiation regimen has been defined. This study aims to compare the efficacy and safety of different treatment regimens and to independently externally validate a recently published reirradiation risk score. We retrospectively analyzed a cohort of patients with recurrent malignant glioma treated with salvage conventionally fractionated (CFRT), hypofractionated (HFRT) or stereotactic radiotherapy (SRT) between 2007 and 2017 at the University Medical Center in Utrecht and Groningen. Moreover, we validated the reirradiation risk score. Of the 121 patients included, 60 patients (50%) underwent CFRT, 22 (18%) HFRT and 39 (32%) SRT. The primary tumor was grade 2/3 in 52 patients and grade 4 in 69 patients with median Overall Survival (mOS) since first surgery of 113 [Interquartile range: 53.2–137] and 39.7 [24.6–64.9] months respectively ($p < 0.01$). Overall, mOS from the first day of re-irradiation was 9.7 months [6.5–14.6]. No significant difference in mOS was found between the treatment groups. In multivariate analysis, Karnofsky performance scale 70% ($p < 0.01$), re-irradiation for first recurrence ($p = 0.02$), longer time interval ($p < 0.01$) and smaller planning target volume ($p < 0.05$) were significant favorable prognostic factors. The reirradiation risk score was validated. In our series, mOS after reirradiation was sufficient to justify more use of this modality. Until a reliable treatment decision tool is developed based on larger retrospective research, the decision for re-irradiation schedule should remain personalized and based on a multidisciplinary evaluation of each patient.

RTHP-18. POST-OPERATIVE RADIOTHERAPY FOR PATIENTS WITH FUNCTIONING AND NON-FUNCTIONING PITUITARY ADENOMAS: A SYSTEMATIC REVIEW AND META-ANALYSIS OF 7551 CASES

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OBJECTIVE: Although surgery is the mainstay of treatment for most pituitary adenomas, post-operative radiotherapy has been shown to be beneficial in improving tumour control and recurrence-free survival. However, due to the potential complications of with radiotherapy, the role of adjuvant radiotherapy in the setting of pituitary adenomas remains unclear. To address this gap, we performed a systematic review and meta-analysis to determine the efficacy and safety of post-operative radiotherapy in the treatment of pituitary adenomas. METHODS: A systematic review was performed according to the Meta-analysis Of Observational Studies in Epidemiology (MOOSE) guidelines. We searched PubMed, MEDLINE and Cochrane databases with no language or publication date restrictions, and included studies where patients were treated post-operatively with any form of radiation therapy. Studies reporting on both functioning and non-functioning pituitary adenomas were included. Outcomes were reported as 5- and 10-year progression-free survival, as well as adverse events rates. Forest plots were generated to determine a pooled event rate and 95% confidence interval (CI) for each outcome using a random effect model analysis. RESULTS: A total of 86 studies from 1986–2017 met the inclusion criteria, with 7551 cumulative patients. Studies included patients with functioning adenomas only (n=12), non-functioning adenomas only (n=12) or both (n=20). The cumulative 5- and 10-year progression-free survival rates were 90.8% (95% CI 86–94%) and 88.6% (95% CI 81–93%), respectively. The overall adverse events rate was 8% (95% CI 5–12%). All outcomes were associated with significant heterogeneity (I2 70%). There were no differences in survival rates or adverse events in relation to study date, tumour pathology, radiosurgery system used or dose of radiation. CONCLUSIONS: Post-operative radiotherapy for pituitary adenomas is effective and safe. Because of the significant heterogeneity and lack of matched controls in the literature, optimum timing and dosage are still unclear. Further prospective studies are needed.

RTHP-20. PEDIATRIC HIGH GRADE GLIOMAS: PATTERNS OF FAILURE AND OUTCOMES WITH LIMITED MARGIN RADIOTHERAPY

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BACKGROUND: The optimal treatment paradigm for pediatric high-grade glioma (HGG) is not clearly defined. Outcomes of patients treated with limited margin conformal radiotherapy (RT) with or without chemotherapy are reported. METHODS: The charts of 39 patients with HGG treated from 2006–2017 were reviewed. In 25 patients, the MRI-defined T2/FLAIR abnormality plus a median 7mm CTV margin was treated to 45–54 Gy, while the T1 post-contrast abnormality plus a median 5mm CTV margin was treated to 57.6–60 Gy. Fourteen patients were treated with single volume approach to 54–59.4 Gy, using a median 7mm CTV margin. Overall survival (OS), progression free survival (PFS), and development of pseudoprogression and radiation necrosis were determined by the Kaplan Meier method. RESULTS: Twenty-nine patients had WHO grade IV disease and 10 had grade III disease. Median age at diagnosis was 11 years (range 4–18). Twenty-four patients underwent subtotal resection, 1 a gross total resection, and 14 biopsy alone. 69.2% of patients received chemotherapy. Median follow-up was 11.7 months. Median PFS and OS for the entire cohort was 8.9 months (95% CI 6.1, 12.2) and 12.2 months (95% CI 9.9, 19.4), respectively. On univariate analysis, only older age was significantly associated with a longer OS (p=0.028). Seventeen patients experienced pseudoprogression and 10 radiation necrosis at a median of 1.1 and 3.8 months after RT, respectively. 38% of patients with pseudoprogression/radiation necrosis were symptomatic, all of whom were treated with steroids. One patient experienced a grade 5 toxicity attributed to brainstem injury. In patients who progressed, 52% experienced an infield failure (90% within the 100% prescription isodose line), and 21% and 27% of patients experienced marginal and distant intracranial failures, respectively. CONCLUSIONS: Outcomes with limited margin RT appear comparable to conventional RT. HGG portends a poor prognosis in the pediatric population and novel treatment strategies are needed.

RTHP-21. CHARACTERIZATION OF RADIATION THERAPY EFFECTS ON CEREBRAL VASCULATURE IN PEDIATRIC BRAIN TUMOR SURVIVORS

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Although cranial Radiation Therapy (RT) plays an important role in the treatment of pediatric brain tumors, it is often associated with significant long-term side effects such as cognitive decline and vascular injury in the form of cerebral microbleeds (CMBs). Understanding the evolution of CMBs and the effect of their presence on surrounding vascular integrity could serve as a risk factor for evaluating the severity of radiation related injury in these children. The goal of this study was to explore the effects of RT on arterial structure, including how arterial thickness changes based on proximity to CMBs of varying sizes. 17 patients (ages 10–24) treated with whole-brain or whole-ventricular RT for pediatric brain tumors 2 months to 16 years prior to imaging, and 3 non-irradiated control patients (ages 14–26) were scanned on a 7 Tesla MRI scanner using a novel simultaneous MRA-SWI acquisition that enables visualization of arteries, veins, and CMBs all on one image. Arterial radii were quantified from segmented vessels and the resulting distribution of vessel radii thickness was correlated with clinical information and CMB burden. Individual CMB size was related to the distance from the nearest vessel. Overall, normalized arterial volume decreased as a function of time since RT. The fraction of small arteries increased with time since RT up to 2 years, after which a decreasing trend was observed. All CMBs were located closer to veins than arteries. Although larger CMBs were initially farther away from the nearest vein, over time CMBs far from surrounding vasculature varied in size. These results suggest that after a CMB forms, the surrounding vasculature narrows and eventually recedes. Current work is investigating the spatial distribution of these findings throughout the brain and how they relate to measured cognitive deficits in these children.

RTHP-22. RADIATION-INDUCED CENTRAL DEMYELINATION, A RARE SUBACUTE COMPLICATION IN 2 CASES

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BACKGROUND: The mechanisms of neurotoxicity from radiation therapy include vascular injury, oxidative damage to neural stem cells and oligodendrocytes, and demyelination, though the pathophysiology is not fully understood. METHODS/PATIENTS: We present 2 cases of an unusual subacute complication of radiation treatment. Case 1: 40-year-old woman with right sided sensorineural hearing loss, progressive gait instability and paresthesias in the right hemiface, diagnosed with a right vestibular schwannoma and treated with surgical resection and fractionated stereotactic radiosurgery. She developed symptomatic central demyelination within the isodose curve of 10 Gy 2 months after radiation, which improved with steroids. Case 2: 26-year-old woman with a right frontal grade II astrocytoma, IDH1 mutant, ATRX loss, treated with resection and external beam radiotherapy (54 Gy in 30 fractions by VMAT). She developed left leg weakness, and MRI evidence of multifocal acute demyelinating lesions within the isodose curve of 10–30 Gy 10 weeks after completing radiation. She improved with high dose steroids and was subsequently able to resume adjuvant temozolomide. None of these 2 patients had a previous history of demyelinating disorder. DISCUSSION: The most frequent acute or subacute complications after radiation therapy are increased contrast enhancement and perilesional edema. Subacute demyelination is a rare entity with only a few cases described in the literature. No prior reported cases have been associated with treatment of vestibular schwannomas with stereotactic radiosurgery. Interestingly in both of the above cases, demyelination occurred within the lower isodose zone ranging from 10–30 Gy. CONCLUSIONS: subacute radiation induced demyelination is a rare complication in brain tumor patients that can be successfully managed with steroids. Demyelination should be on the differential diagnosis for new distant enhancing lesions following radiation in the primary brain tumor population.

RTHP-23. HEARING AND FACIAL PRESERVATION FOR VESTIBULAR SCHWANNOMAS TREATED WITH STEREOTACTIC RADIOSURGERY OR FRACTIONATED STEREOTACTIC RADIOTHERAPY

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BACKGROUND: Vestibular schwannomas (VS) are benign intracranial neoplasms arising from the eighth cranial nerve. In recent years, advancing radiological techniques have provided successful non-invasive alternatives for VS therapy. RESEARCH QUESTION: How do the long-term clinical outcomes vary for the three current radiation modalities of stereotactic radiosurgery (SRS), fractionated stereotactic radiotherapy (FSRT), and hypofractionated stereotactic radiotherapy (hypoFSRT)? METHODS: A retrospective chart review was conducted for all patients with unilateral VS at a single academic medical center from 2000 to 2017. Audiograms, physical examinations, and patient-reported symptoms were extracted to assess hearing, facial function and sensation, and statuses of tinnitus, vertigo, and imbalance. RESULTS: Sixty patients were identified (33 FSRT cases, 21 SRS cases, and 6 hypoFSRT cases). Post-operative onset of vertigo and tinnitus demonstrated significant difference between the three modalities (p = 0.035, 0.005). Survival rate analysis revealed the survival time to tinnitus onset also varied significantly when comparing SRS and FSRT (p = 0.050). SIGNIFICANCE: This study aims to elucidate the hearing and facial preservation of the three radiological interventions, thus enabling physicians to optimize patient care.

RTHP-24. CLINICAL FEATURES OF BASAL GANGLIA GERM CELL TUMORS AND ITS CLINICAL OUTCOMES

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OBJECTIVE: To provide a basis for standardizing the diagnosis and treatment of basal ganglia germ cell tumors (GCTs) by summarizing the clinical features and sharing our clinical experiences. METHODS: Of 320 patients with intracranial GCTs treated in our hospital from 2005 to 2017, 37 developed basal ganglia GCTs, including 34 male and 3 female, with a median age of 12 years. The symptoms included extremity weakness, headache, precocious puberty, diabetes insipidus and hypoevolutism. The lesions predominately located in left basal ganglia. Two patient was diagnosed as having spinal metastasis. Positive serum tumor markers was found in 22 cases. Tumor markers detection for 15 cases of cerebrospinal fluid showed 7 of -HCG (+) and 1 of -HCG (+) and AFP (+). Seventeen patients were pathologically diagnosed as GCTs (n=13), yolk sac tumor (n=2), embryonal carcinoma (n=1) and immature teratoma (n=1); 17 were diagnosed with tumor markers; and 3 received diagnostic radiotherapy. Surgery was conducted in 17 cases. Thirty-six patients received whole CNS radiotherapy and local radiotherapy, and the other 1 underwent chemotherapy. Adjuvant chemotherapy included BEP in 22 cases and EP in 8 cases. Tumor markers, imaging features and survival time were evaluated. RESULTS: Due to incomplete imaging data, 4 of 37 cases weren't evaluated. Of 33 cases, CR was achieved in 16 cases; PR in 13; SD in 1; and PD in 3. After being followed-up to October 2017, 27 patients were alive, 7 were lost and 3 died. The 5-year progression-free survival rate was 88.8%. CONCLUSION: Basal ganglia GCTs is common in male, frequently involving left basal ganglia, with good prognosis. Chemoradiotherapy are suggested for patients with -HCG and/or AFP (+). For those with -HCG and/or AFP (-), pathologic diagnosis should be performed before chemoradiotherapy, and diagnostic radiotherapy is not recommended for its high risk.

RTHP-25. THERAPEUTIC EFFECT OF SRS IN 34 CASES OF BRAIN METASTASES OF NSCLC WITH A MAXIMUM DIAMETER ≥ 4CM AND ANALYSIS OF PROGNOSTIC FACTORS

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OBJECTIVE: To retrospectively summarize the therapeutic effect of SRS in 34 cases of brain metastases of NSCLC with the maximum diameter 4 cm, and discuss the prognostic factors. METHODS: From 2006 to 2015, 34 patients suffering from brain metastases of NSCLC with the largest diameter 4cm were analyzed. Patient information were analyzed, including gender, age, pre-treatment KPS, RPA grade, primary tumor types, EGFR mutation types, extracranial metastasis, metastatic position, number of metastases, total lesion volume, maximum lesion volume, whether receiving surgery or combined WBRT, with or without chemotherapy after radiotherapy, and radiotherapy dose. Imaging evaluation was performed at 3 months after treatment. The survival rate was analyzed with SPSS 17.0 software, and Kaplam-Meier method was used to calculate survival rates which were then compared by log-rank test. RESULTS: MRI images were missed in 5 cases. Of the other 29 cases, CR was achieved in 6 cases; PR in 13; SD in 4; and PD in 6. The median progression-free survival and total survival were 6 and 16 months, respectively. One and 2 survival rates were 37.3% and 67.5%, respectively. Univariate analysis showed that KPS, RPA grade, number of metastases, with or without surgery, whether receiving adjuvant chemotherapy or TKI had no significant correlation with survival prognosis. The maximum lesion volume was less than 28.3 cc, which is considered as a possible prognostic factor. CONCLUSION: SRS (FSRS) is feasible for brain metastases of NSCLC with the maximal diameter 4 cm, achieving a therapeutic effect which wasnt inferior to WBRT or 3D-CRT or SRS. No significant correlation was found between KPS, RPA grade, metastatic lesions and prognosis. The maximum lesion volume less than 28.3cc may be a favorable prognostic factor. More cases are needed to further observe the therapeutic effect.

RTHP-26. ANALYSIS OF OUTCOMES FROM RE-IRRADIATION WITH PULSED REDUCED DOSE RATE RADIATION THERAPY FOR HIGH GRADE GLIOMAS

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BACKGROUND: Treatment options for recurrent high-grade glioma are limited. We updated a retrospective series of treatment completed pulsed reduced dose rate radiation therapy (PRDR), a method to decrease toxicity and improve efficacy. METHODS: 103 patients with glioblastoma (GB) and 45 patients with grade 3 gliomas were analyzed (12/2000 to 12/2016). 29 GBs and 16 grade 3 gliomas had transformed from lower grade gliomas. Treatment was with at an apparent dose rate was 6.67 cGy/min. Median re-irradiation dose was 54 Gy (44–54 Gy) with median total dose of 114 Gy (range 69–171.4 Gy). Five GBs and seven grade 3's received whole brain PRDR. Kaplan Meier method was used to analyze survival. Univariate and multivariate analysis (MVA) used a Cox proportional hazards model. RESULTS: Median survival from initiation of PRDR was 7.1 months in post-Avastin GBs and 6.0 months in Avastin naive GBs. Survival was 7.8 months for grade 3's from PRDR. Survival from treatment at first progression was 11.9 months in both the Avastin naïve GBs and Avastin treated GBs while grade 3's survived 16.7 months. A partial response was observed in14.4% of GBs and 20% of grade 3's while stable disease was seen in 16.5% of GBs and 22% of grade 3's. Therapy was well tolerated. Toxicity data and MVA analysis, which includes: time from initial to re-irradiation, KPS, transformation from lower grade histology, whole brain PRDR, tumor location, planning treatment volume size, MGMT status, IDH1 status, 1p/19q status, and age will be presented. CONCLUSIONS: In comparison to historical data these results are encouraging. A phase II clinical trial is ongoing to confirm these results prospectively.

RTHP-27. PATTERNS OF FAILURE AFTER STEREOTACTIC RADIOSURGERY FOR RECURRENT HIGH-GRADE GLIOMA: A SINGLE INSTITUTION EXPERIENCE OF 10 YEARS

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BACKGROUND: Stereotactic radiosurgery (SRS) is a treatment modality that is frequently used as salvage therapy for small nodular recurrent high grade gliomas (HGG). Due to the infiltrative nature of HGG, it is unclear if this highly focused technique provides a durable local control benefit. Objective To determine how demographic or clinical factors influence the pattern of failure following SRS for recurrent high grade gliomas. METHODS: We retrospectively reviewed clinical, radiographic and follow-up information for 47 consecutive patients receiving SRS for recurrent HGG at our institution between June 2006 and July 2016. All patients initially presented with a HGG (WHO Grade III and IV). Following SRS for recurrence, all patients experienced treatment failure, and we evaluated patterns of local, regional, and distant failure in relation to the SRS 50% isodose line. RESULTS: Most patients with recurrent HGG developed in-field treatment failure following SRS (N = 40; 85%). Higher SRS doses were associated with longer time-to-failure (HR = 0.80 per 1Gy increase; 95% CI 0.67–0.96; P = .016). There was a statistically significant increase in distant versus in-field failure among older patients (P=.035). This effect was independent of bevacizumab use (OR = 0.54, P =1.0). CONCLUSIONS: Based on our experience, the majority of treatment failures after SRS for recurrent HGG were in-field. Older patients, however, presented with more distant failures. Our results indicate that higher SRS doses delivered to a larger area as fractioned or unfractioned regimen may prolong time-to-failure, especially in the older population.

RTHP-28. REPEAT SALVAGE GAMMA KNIFE RADIOSURGERY FOR RECURRENT GLIOBLASTOMA

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BACKGROUND: Our institutional practice has been to treat recurrent glioblastoma (GB) with gamma knife radiosurgery (GK). We evaluated survival outcomes in these patients. METHODS: This was a single institution review of patients with GB who underwent a definitive course of postoperative external beam radiotherapy (EBRT) with concurrent chemotherapy and subsequently treated with GK for recurrence or progression between 2006 and 2017. Overall survival (OS) was determined using Kaplan-Meier statistics. RESULTS: 87 patients were identified with median age of 55 years. Median follow-up was 21.1 months. The majority of patients underwent gross total (57%) or subtotal resection (38%). 23 (26%) underwent surgery for recurrence. The median marginal dose for first GK was 17 Gy and 14 Gy for patients with 1 target or >1 target, respectively. 4 patients underwent fractionated GK treatment with 18–25 Gy in 3–5 fractions. 20 patients underwent a second GK treatment and 1 patient was treated three times. Time from first to second GK was 6 months, with a median marginal dose for second or third GK of 15 Gy. 10 patients underwent a second course of EBRT ranging from 30–59.4 Gy in 10–33 fractions; 2 patients received stereotactic body radiation therapy with 25 Gy in 5 fractions. Median OS was 21.4 months. 2- and 5-year OS was 41.8% and 9.9%, respectively. Median survival after first and second GK was 9.6 months and 4.7 months, respectively. There was a trend towards improved OS for patients treated with both EBRT and GK compared to GK alone (Median OS: 2.85 years vs 1.75 years, p=0.14). DISCUSSION: To our knowledge, this study reports on the largest cohort of patients treated with salvage GK for recurrent GB. The survival outcomes compare favorably to contemporary trials. Future studies will include an analysis of toxicity and patterns of failure.

RTHP-29. A FEASIBILITY STUDY OF RADIATION THERAPY DOSE ESCALATION GUIDED BY SPECTROSCOPIC MRI IN PATIENTS WITH GLIOBLASTOMA

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The standard of care for glioblastoma is neurosurgical resection followed by radiation therapy (RT) and temozolomide (TMZ) chemotherapy. Previous RT with TMZ dose escalation attempts with RT doses up to 75 Gy targeted gadolinium contrast enhancing portions of tumor. These studies generated no significant benefits in recurrence or survival, but this may be because areas of gadolinium contrast enhancement only partially correlate with areas of future recurrence. Proton spectroscopic MRI (sMRI) measures endogenous metabolite levels without intravenous contrast agent. Elevated choline to N-acetylaspartate ratios (Cho/NAA) measured with whole brain sMRI mapping have significantly correlated with histological disease and, intriguingly, sMRI metabolic abnormalities predate disease recurrence. To enable RT dose escalation by sMRI in a multi-institutional clinical study setting, we specifically designed a cloud software platform to incorporate sMRI into the RT planning workflow. In order to test the sMRI workflow across institutions, a sMRI-guided dose escalation feasibility study is underway at three university hospitals (Emory, Johns Hopkins, and U. Miami). Eligible patients with glioblastoma after biopsy or surgical resection undergo MRI and sMRI within 14 days of start of therapy with TMZ and dose painted RT. Elevated Cho/NAA and residual contrast-enhancing lesions are targeted for boosted radiation to 75 Gy while the remaining tissue received standard-of-care therapy to 60 or 51 Gy in 30 fractions. Patients also receive a second sMRI scan during the third week of therapy to assess for early tumor response. Preliminary results are encouraging. In the first of three years of planned accrual, 11 patients (Emory 5, Miami 4, Hopkins 2) of a planned 30 have been treated within this trial with no observed serious adverse events. As this is a technical feasibility study with no control group, we plan for a future randomized cooperative group study with sMRI dose escalation versus standard of care.

RTHP-30. MANAGEMENT OF GLIOBLASTOMA IN THE ELDERLY - A 10-YEAR ANALYSIS OF THE BC CANCER AGENCY POPULATION

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PURPOSE: Glioblastomas (GBM) are the most common primary tumours of the brain and carry an extremely poor prognosis. Despite recent publications, management of the elderly remains heterogeneous. We investigate the impact of patient characteristics and treatment modalities on the outcome of elderly patients with GBM in British Columbia (BC) from 2005–2015. METHODS: Using the BC Cancer Agency patient registry, 822 adults diagnosed from 2005–2015, age 60 with histologically confirmed GBM ICD-O-3 codes (9440/3, 9441/3, 9442/3) were identified. Univariate, multivariate and Kaplan-Meier analyses were performed on patient characteristics and treatment modalities for overall survival (OS). RESULTS: Median OS was 6.57 months (0.03–99.93). 60% of the patients were male, mean age at diagnosis was 70 (60–90). Patients were aged 60–64 (26%), 65–69 (27%), 70–74 (20%), 75 (27%). 65% of the cohort was diagnosed between 2011–2015 vs. 35% between 2005–2010. 96% of patients had a diagnosis of glioblastoma with the remainder giant cell glioblastoma and gliosarcoma. *MGMT* promoter methylation status was available in 11% of patients. Resection status was GTR/STR (72%), biopsy (28%). 41% of patients received chemotherapy, 77% of patients received radiation therapy (RT). 20% of patients did not receive treatment beyond surgical intervention. Patient management involved concurrent chemoirradiation (CRT) (40%), RT alone (37%), chemo alone (1.5%). Younger patients aged 60–69, concurrent CRT and maximal safe resection (GTR/STR) resulted in improved OS (p<0.0001). Year of diagnosis and RT dose fractionation (60/30 (38%) vs. 40/15 (36%)) were not associated with a difference in OS. CONCLUSION: Elderly patients with GBM treated in BC 2005–2015 achieved similar outcomes to those of existing published data. Younger age, aggressive resection and concurrent CRT were associated with improved OS. Ongoing analysis is directed at further correlation of patient and treatment factors, features of RT and chemotherapy administration, progression-free survival and patterns of failure.

RTHP-31. STEREOTACTIC RADIOSURGERY FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA

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INTRODUCTION: Primary central nervous system lymphoma (PCNSL) is rare, with a treatment backbone that typically includes high-dose methotrexate. Whole brain radiotherapy (WBRT) is often reserved for patients who cannot receive chemotherapy, or with residual/recurrent disease, and demonstrates favorable local control however with significant toxicity. We report outcomes of stereotactic radiosurgery (SRS) in patients with PCNSL. METHODS: We performed a retrospective single-institution review to identify 21 patients with PCNSL treated with SRS between 1998 and 2017. The Kaplan-Meier method was used to describe overall survival (OS) and freedom-from distant brain progression (FFDBP). RESULTS: The median age at diagnosis was 66 years (IQR 55–73), and the median age at SRS was 67 years (IQR 56–75). The median KPS at SRS was 80 (IQR 50–80). Nineteen patients (90%) received methotrexate-based chemotherapy prior to SRS (median of 8 cycles). Eleven patients (52%) received SRS for recurrent disease, while 8 patients (38%) underwent SRS for persistent disease after chemotherapy. The median dose was 15 Gy in 1 fraction or 23 Gy (IQR 22.5–25 Gy) in 3–5 fractions. The median PTV was 3.3 cc (IQR 1.5–19.8cc). Local control at 3 and 6 months was 93% and 92% respectively, however 3 month FFDBP was 30%. OS at 3 and 6 months was 67% and 38%, respectively. Local control was higher in patients who received pomalidomide or lenalidomide after SRS (p = 0.035). Consolidative etoposide and cytarabine was associated with longer time to progression after SRS (p = 0.032) and improved OS at 6 months (p = 0.017). CONCLUSION: SRS offers effective local tumor control, however prognosis remains poor due to a high rate of distant progression after treatment. SRS may have a role in the salvage setting for patients with recurrence after WBRT, or allow deferral of WBRT, however systemic therapy may most strongly influence survival outcomes.

RTHP-32. RECONSIDERING THE PROGNOSTIC IMPACT OF AGE, GRADE, AND EXTENT OF RESECTION ON CLINICAL OUTCOMES OF 1p/19q CODELETED OLIGODENDROGLIOMAS AFTER RADIATION THERAPY: A MULTI-INSTITUTIONAL REPORT

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OBJECTIVE: Age, grade, and extent of resection (EOR) are key factors to guide the treatment of gliomas. This multi-institutional study examines their prognostic impact on the favorable subset of molecularly-defined oligodendroglioma after definitive radiation therapy (RT). METHODS: Grade 2–3 oligodendroglioma patients treated with RT with or without chemotherapy from 2000–2017 at four tertiary academic cancer centers were retrospectively reviewed. Eligible patients were required to have 1p/19q codeletion as well as defined grade and EOR within 12 months of RT. Progression-free survival (PFS) and overall survival (OS) rates were determined using Kaplan-Meier analyses. Cox regression analysis was used to identify factors associated with worse PFS and OS. RESULTS: One hundred sixty-eight patients were identified with median follow-up of 43 months (0.5–200). Median age was 46 (23–80), 79(47%) had gross-total resection, 121(72%) were grade 3; 106(63%) received adjuvant RT and temozolomide (TMZ), 26(15%) received adjuvant RT and procarbazine/lomustine/vincristine (PCV), 18(11%) received adjuvant RT alone, and 18(11%)

received prior chemotherapy before salvage surgery and RT. Overall, the 10-year PFS and OS were 52% and 65%, respectively. On multivariable analysis, lower KPS (HR:0.96, 95% CI:0.93–0.99), gliomatosis (HR:195, 95% CI:16–2340), and prior chemotherapy (HR:2.2, 95% CI:1.04–4.7) were the only significant factors associated with worse PFS; notably, age, grade, and EOR were not significant for PFS. In a subset of 146 patients excluding those with gliomatosis and prior chemotherapy, age >40, grade 3, subtotal resection, TMZ chemotherapy, and lower RT dose were not associated with worse PFS or OS rates (all p >0.05). CONCLUSIONS: Oligodendrogliomas with 1p/19q codeletion have favorable outcomes after RT, except those with gliomatosis or prior chemotherapy exposure. Traditional prognostic factors of age >40, EOR, and grade do not appear to reliably risk stratify these patients after adjuvant RT. Their use to guide treatment selection may need to be revisited.

RTHP-33. APPLICATION OF PRESURGICAL NAVIGATED TRANSCRANIAL MAGNETIC STIMULATION MOTOR MAPPING FOR ADJUVANT RADIOTHERAPY TREATMENT PLANNING IN PATIENTS WITH BRAIN TUMORS

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BACKGROUND: Lately navigated transcranial magnetic stimulation (nTMS) has been implemented in the neurosurgical routine to detect motor-eloquent areas for safe and complete resection of high-grad gliomas (HGG). However, in radiotherapy (RT) planning, the primary motor cortex is not respected in target volume delineation. This study evaluates the implementation of nTMS motor mapping for RT planning in patients harbouring motor-eloquent HGGs. METHODS: nTMS motor maps of 30 patients diagnosed with motor-eloquent HGGs were fused with RT planning and imaging and volumetric modulated RT plans were optimized using nTMS maps as organ at risk (OAR). Doses to nTMS motor maps were evaluated using dose-volume histogramm (DVH) parameters. RESULTS: Mean dose (Dmean) to the nTMS motor maps was 42.3 Gray (Gy) (3.7–61.1 Gy) and was significantly reduced by 14.3% to 37.0 Gy (3.6 - 55.8 Gy, p<0.05) when constraining the dose to nTMS motor areas to 45 Gy. Yet, the dose to the planning total volume (PTV) was not compromised. Even with an additional dose escalation of 70 Gy to the tumor area, nTMS motor maps can be spared by 4.6 +/- 3.5 Gy (12.8%, p<0.05). CONCLUSIONS: nTMS motor maps can be easily implemented in standard RT planning and applied for target contouring in the treatment of HGGs. Doses to motor-eloquent areas can be significantly reduced when considering nTMS motor maps without affecting treatment doses to the PTV. Thus, nTMS could be used as a valuable tool in RT planning.

RTHP-34. CRANIOSPINAL IRRADIATION (CSI) AS PART OF RE-IRRADIATION (RT2) FOR CHILDREN WITH RECURRENT INTRACRANIAL EPENDYMOMA

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PURPOSE: To evaluate outcomes in children with relapsed intracranial ependymoma who received focal initial radiotherapy (RT1) and subsequently underwent RT2 with or without CSI. METHODS: 32 patients under age 18 were identified in this retrospective review. Patients with distant relapse received craniospinal irradiation (CSI) as part of RT2. Prior to 2012, those with local relapse were treated with 54 Gy focal RT2 (1.8 Gy/fraction). From 2012 onwards, our practice changed to include 23.4–36 Gy CSI, followed by boost RT2 to all sites of resected or gross disease (CSI+RT2). The Kaplan-Meier method was used to calculate overall survival (OS) and freedom-from-progression (FFP); groups were compared using the log-rank test. RESULTS: Median follow-up was 5.5 years. Of 10 patients with distant relapse after RT1, 2-year OS and FFP were 66.7% (95% CI 28.2–87.8) and 22.2% (95% CI 3.4–51.3); amongst this group, there were no 5-year survivors. Of 22 patients with local-only recurrence after RT1, use of CSI (n = 7, median age 8.4 at RT2) was associated with a statistically significant improvement in FFP (5-year probability 83.3% [95% CI 27.3–97.5] with CSI+RT2 vs. 15.6% [95% CI 2.6–38.7] with focal RT2, p = 0.025). In those with posterior fossa (PF) ependymoma and local-only recurrence after RT1 (n = 16), 5-year FFP was 100% (95% CI 100-100) with CSI+RT2 vs. 10.4% (95% CI 0.6–36.4, p = 0.034) with focal RT2. There was no OS difference between those who received CSI+RT2 vs. focal RT2 (5-year OS; all tumour locations: 80.0% [95% CI 20.4–96.9] vs. 43.1% [95% CI 17.9–66.2], respectively, p = 0.24; PF subgroup: 100% [95% CI 100-100] vs. 36.7% [95% CI 11.3–63.0], respectively; p = 0.093). There were no cases of radiation necrosis attributable to RT2. CONCLUSION: Re-irradiation with CSI is a safe and effective approach to control recurrent disease in children with ependymoma.

RTHP-35. SURVIVAL IMPACT OF DELAYS PROLONGING THE OVERALL DURATION OF ADJUVANT RADIATION THERAPY FOR PATIENTS WITH GLIOBLASTOMA

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BACKGROUND: Though conventionally fractionated chemoradiation (CRT) is well tolerated by selected patients with newly diagnosed glioblastoma (GBM), both adverse health related and non-health related factors can lead to unplanned interruptions in treatment. The effects of prolonged time to completion (TTC) of radiation therapy (RT) on overall survival (OS) for these patients are unclear. METHODS: The National Cancer Database (NCDB) was queried for all adult patients with newly diagnosed GBM undergoing surgical resection followed by adjuvant CRT with conventionally fractionated RT (6000 - 6600 cGy in 30 - 33 fractions) from 2005 to 2012. TTC was defined as the interval from first to last fraction of RT. Recursive partitioning analysis (RPA) was used to determine a threshold for TTC of adjuvant RT. Cox proportional hazards modeling was used to identify covariates associated with OS. RESULTS: A total of 13,489 patients were included in our cohort. Patients who completed adjuvant RT within the RPA-defined threshold of 46 days from initiation of RT (median OS: 14.0 months, 95% CI 13.7 - 14.3 months) had significantly improved OS compared to patients with TTC of 47 days or greater (median OS: 12.0 months, 95% CI 11.4 - 12.6 months, p<0001). CONCLUSIONS: Delays in completing adjuvant radiation therapy were associated with a worse survival outcome. Any unnecessary delays in completing adjuvant RT should be minimized while ensuring the safe delivery of therapy.

RTHP-37. IMPACT OF ^{11}C-METHIONINE/FDG DURAL TRACER PET-BASED, COMPARED WITH MRI-BASED TARGET DELINEATION OF MALIGNANT GLIOMAS FOR RADIATION PLANNING

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Precise delineation of target volume for radiotherapy of malignant glioma is one of the crucial processes in glioma treatment. Although MRI is the modality often chosen for this procedure, the authors' previous research showed that combined use of 11C-methionine (MET) and 18F-fluorodeoxyglucose (FDG) PET was more accurate for glioma visualization (J Nucl Med, 2012). The aim of this study was to analyze volumetric and geometrical properties of RT-target-delineation based on MET/FDG PET and to compared them with those based on MRI. The impact of geometrical properties on progression free survival was further investigated. Twenty-five patients with a diagnosis of malignant glioma were included in our study. Three target volumes were compared, which included contrast-enhancing core lesions identified by contrast-enhanced T1 weighted images (T1Gd), high intensity lesions on T2 weighted images, and lesions showing high DS, a cell density score calculated from MET/FDG PET (> 3; hDS). T1Gd with 2.0 cm margin was able to cover the entire hDS only in 6 (24%) patients, indicating microscopic invasion of glioma cells beyond 2cm from gadolinium-enhanced core. Insufficient coverage of high DS region with RT target volume was suggested to be a risk for out-field recurrence. Higher coverage of hDS (T1Gd + 2/hDS) by T1Gd with 2 cm margin tended to

positively impact overall and progression free survival. Cox regression analysis demonstrated that low coverage of hDS by T1Gd with 2 cm margin was predictive of tumor recurrence outside gadolinium-enhanced core, indicative of out-field reoccurrence. Present findings indicate that MRI is inadequate for target delineation for radiation therapy in malignant glioma treatment. Expanding additional margins to MRI based target volume often reduces this uncertainty while unnecessarily irradiated regions increase and MET/FDG PET seemed to provide more accurate information for target delineation than MRI.

RTHP-38. BNCT COMBINED WITH EARLY SUCCESSIVE BEVACIZUMAB TREATMENTS FOR RECURRENT MALIGNANT GLIOMAS

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INTRODUCTION: Recurrent malignant gliomas (RMGs) are difficult to control. We have often treated RMGs using tumor-selective particle radiation called boron neutron capture therapy (BNCT). However, despite the cell-selectivity of BNCT, brain radiation necrosis (BRN) may develop. This is partly due to the full-dose X-ray treatments usually given earlier in the treatment course. To overcome BRN, we herein used extended BV treatment beginning just after BNCT, and evaluated the feasibility, efficacy, and BRN control of this combination treatment. METHODS: Two cohorts were included. The first and second cohorts were treated with BNCT between June 2013 and May 2014, and between August 2017 and December 2017, respectively. They were followed by successive BV treatments. The first cohort was composed of 7 patients with RMGs (4 grade 4 and 3 grade 3 cases). The second cohort was composed of 6 patients with RMGs (5 grade 4 and 1 grade 3 cases). They were followed-up to April 2018. RESULTS: Median OS and PFS after combination treatment were 15.1 and 5.4 months, respectively in the 1st cohort. Those in the second cohort were not reached. The OS data was compared with RPA classification for RMG as advocated by Carson et al. in a 2007 article in JCO. In both cohorts no symptomatic aggravation of BRN occurred if BV could be continued. CTCAE grade 2 and 3 proteinuria occurred in two cases and necessitated the interruption of BV treatments. Totally 11cases' OS in both cohorts were compared using JCO's RPA classification. Nine out of 11 cases showed longer OS in comparison with corresponding JCO's RPA classes. CONCLUSION: BNCT followed by BV treatments well-prevented or well-controlled BRN with prolonged OS and acceptable incidence of adverse events in our patients with RMG.

RTHP-39. SINGLE ARM, MULTI-CENTRIC PHASE II CLINICAL STUDY NAMED "BORON NEUTRON CAPTURE THERAPY AND TEMOZOLOMIDE IN TREATING PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA MULTIFORME"

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INTRODUCTION: We have applied tumor-selective particle radiation boron neutron capture therapy (BNCT) to malignant brain tumors. Here we introduce the single arm, multi-centric phase II clinical study named "BNCT and Temozolomide in Treating Patients with Newly Diagnosed Glioblastoma Multiforme" (OSAKA-TRIBRAIN0902, NCT00974987). MATERIALS AND METHODS: Nine neurosurgical institutes and a neuclear reactor were involved in this trial. From September 2009 to January 2014, 32 newly diagnosed GBMs were enrolled. Primary end point was overall survival 2 years (OS2yr) and the results were compared with Stupp's ones published in NEJM 2005. The secondary endpoints were local control rate and incidence of brain radiation necrosis. We applied BNCT with 2 boron compounds, BSH and BPA simultaneously. Limiting factor for BNCT was setted for the maximum normal brain dose as 13 Gy-Eq. BNCT was followed by conventional XRT with three layers gradient of 8, 16, 24 Gy as opposing portal fashion from brain surface to the bottom of tumor clinical target volume. This was aimed to compensate BNCT's heterogeneity and shortage of deep tumor absorbed dose and decrease the possibility of brain radiation necrosis (BRN). The patients were administered with TMZ as Stupp's regimen as long as possible. RESULTS: At the initial plan, patients' registration was scheduled as 45 cases, however we had to finish patients accrual as 32 cases due to the disastrous earthquake in Japan. OS2yr of this clinical trial adjusted by Kaplan-Meyer analysis was 45.5% (29.9 - 59.9, as confidence interval), which is superior to historical control, Stupp's published OS2yr 27.3% (p=0.012). Local control was achieved in 64.3% (35.1–87.2) and cumulative incidence of BRN was 7/28 cases (25%). DISCUSSIONS: In this study, BNCT followed by XRT/TMZ exceeded the results of standard treatment (XRT/TMZ) in OS with acceptable BRN incidence and excellent local tumor control.

RTHP-40. ENDOCRINE AND METABOLIC ABNORMALITIES IN INTRACRANIAL GERM CELL TUMOR PATIENTS TREATED WITH IRRADIATION

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OBJECTIVE: To evaluate the endocrine and metabolic abnormalities in intracranial germ cell tumor (iGCT) patients treated with irradiation. PATIENTS AND METHOD: Seventy-seven iGCT patients who had received radiotherapy and endocrine follow-up in Huashan Hospital between January, 2010 and July, 2017 were retrospectively analyzed, consisting of forty-nine germinomas and twenty-eight NGGCTs. The median doses for the whole brain, spine and primary tumor site were 30.6Gy, 30.6Gy and 50.0Gy. The median follow-up period was 50.0 months. Fifty-one patients had radiologically proved suprasellar/sellar lesions. Results: The 5-year overall and recurrence-free survival rates were both 98.7%. The prevalence of central adrenal insufficiency (CAI), central hypothyroidism (CHT), central hypogonadism (CHG), hyperprolactinemia (HPL) and central diabetes insipidus (CDI) in those with radiologically proved suprasellar/sellar lesions was 71.4%, 70.0%, 73.5%, 48.4% and 70.2%, while that in patients without suprasellar/sellar lesions was 30.8%, 28.0%, 25.9%, 15.0% and 16.7% separately. Compared to that before irradiation, CAI, CHT and CHG wasn`t significantly improved while the overall level of prolactin and prevalence of CDI declined significantly (P=0.03 and 0.001). The irradiation dosage wasn`t significantly associated with the prevalence of CAI, CHT and CHG. Based on BMI, 30.4% were overweight and 3.0% were obesed in patients below 18 years old. 72.1% patients had dyslipidemia, with the abnormality of triglyceride most commonly. One patient developed diabetes mellitus after irradiation. The prevalence of hyperuricemia was 50.0%. The irradiation dosage wasn`t associated with BMI, triglyceride, total cholesterol LDL-c, HDL-c, HOMA-IR or serum uric acid level. CONCLUSION: The prevalence of CAI, CHT, CHG, HPL and CDI was high in iGCTs patients, and those without suprasellar/sellar lesions may develop those endocrine abnormalities too. The post-irradiation level of prolactin and prevalence of CDI declined significantly. Metabolic abnormalities including overweight, dyslipidemia, and hyperuricemia were common in iGCTs patients. The irradiation dosage wasn`t associated with occurrence of hypopituitarism and those metabolic parameters.

RTHP-41. LONG-TERM STROKE RISK IN MENINGIOMA PATIENTS TREATED WITH CONVENTIONALLY FRACTIONATED PHOTON-BASED RADIATION THERAPY

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INTRODUCTION: Fractionated radiotherapy (RT) has been a stalwart treatment modality for intracranial meningioma for decades, providing excellent tumor control. The increasing access to proton therapy and theoretical advantages based on physical properties are likely to lead to increased number of patients with meningioma and other intracranial tumors receiving proton-beam RT. A recent randomized study of RT from the Massachusetts General Hospital involving an 80% to 20% proton/photon ratio during treatment for subtotally resected/recurrent benign meningioma patients found the risk of stroke was 20.5%, with the average stroke developing 5.6 years after RT completion (Sanford et al., 2017). The long-term stroke risk in patients with meningioma treated with photon-based fractionated RT has not been previously studied. METHODS: A PubMed database search for relevant articles examining photon-based RT for meningioma with minimum mean/median follow-up of six years was undertaken. Stroke rate was assessed either from direct description in manuscripts, or from extrapolating post-RT complications from reported clinical examinations (i.e. hemiparesis/weakness, pituitary dysfunction following treatment of cavernous sinus lesions). Results were then culled to determine an overall stroke rate. RESULTS: Six studies met inclusion criteria; 303 patients received photon-based RT for meningioma with a sufficient long-term follow-up. Median/mean follow-up ranged from 78–168 months. Operative resection prior to RT occurred in 213 patients (70.3%). Seventeen patients suffered a stroke following RT, yielding a rate of 5.6%. CONCLUSIONS: The long-term stroke risk in patients with meningioma treated with photon-based conventionally fractionated RT appears to be significantly lower than the stroke rate reported in a randomized phase III trial utilizing a 80/20 combination of protons and photons. Future studies are necessary to determine if photon-based therapy may actually be safer for patients with intracranial diseases, such as benign meningioma.

RANDOMIZED BRAIN TUMOR TRIALS IN DEVELOPMENT

RBTT-01. RANDOMIZED PHASE 2 OPEN LABEL STUDY OF NIVOLUMAB PLUS STANDARD DOSE BEVACIZUMAB VERSUS NIVOLUMAB PLUS LOW DOSE BEVACIZUMAB IN RECURRENT GLIOBLASTOMA

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BACKGROUND: The outcome for glioblastoma (GBM) remains dismal with a median survival of 15 months. Vascular endothelial growth factor (VEGF) is a highly upregulated proangiogenic growth factor in GBM that contributes to tumor-associated immunosuppression by inhibition of dendritic cell maturation and antigen presentation, induction of apoptosis of CD8+ T cells and enhancing Treg activity. Hence, a combination of anti PD1 and anti VEGF is promising approach in recurrent GBM. Lower anti-VEGF therapy dosing can lead to enhanced immune infiltrate and improved survival following co-administration with an anti-tumor immunotherapeutic in preclinical studies. METHODS: This is a 90 patient randomized phase 2 open label study of nivolumab plus standard dose bevacizumab versus nivolumab plus low dose bevacizumab in recurrent GBM. Primary endpoint is to evaluate the efficacy of nivolumab when administered with standard and reduced dose bevacizumab as measured by overall survival (OS) at twelve months (OS-12). Secondary endpoint include safety, Progression free survival at 6 months, OS and overall response rate. Exploratory endpoints include circulating immunologic biomarkers, cytokines, archival tumor PD-L1 expression and inflammatory gene expression signature and perfusion and diffusion weighted imaging with response (RANO and iRANO). Eligibility Criteria include Age ≥ 18 years, first recurrence of GBM, normal organ function, KPS ≥ 70. Key exclusion criteria include active, known or suspected autoimmune disease, contraindications for bevacizumab therapy and decadron > 4 mg/day or equivalent of steroids. STATISTICAL ANALYSIS: The one-sample log-rank test will be applied to outcomes observed for each arm individually to test the hypothesis that OS has been improved beyond the null 12-month survival rate of 45%. With N=45 patients per arm, a one-sided test provides power=0.80 to detect survival rate of 58% at 12-months following treatment at the 0.10 significance level. Results: The study (NCT03452579) is ongoing and enrolling GBM patients in first recurrence.

RBTT-02. ENHANCING VACCINE RESPONSES WITH DOSE-INTENSIFIED TEMOZOLOMIDE IN GLIOBLASTOMA: INITIATION OF THE I-ATTAC TRIAL

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Two of our previous clinical trials targeting Cytomegalovirus (CMV) protein pp65 in newly diagnosed glioblastoma (GBM) using pp65-specific dendritic cell (DC) vaccines have yielded long term survival rates greatly exceeding those predicted with standard of care. In the ATTAC trial (IND 12839), patients received sequential DC vaccination throughout monthly cycles of standard temozolomide (STD-TMZ) 200mg/m^2/d for 5d and were randomized to one of two vaccine site preconditioning regimens. Significantly higher rates of DC migration (p=0.04) and survival (p=0.013) were observed in patients randomized to tetanus preconditioning, with half of the cohort living to nearly five years after diagnosis. In the ATTAC-GM trial, we treated a subsequent cohort with dose-intensified TMZ (DI-TMZ) 100mg/m^2/d for 21d prior to and throughout vaccination with GM-CSF-containing DC vaccines and observed extraordinarily prolonged progression-free survival (PFS) and overall survival (OS) (median 25.3 and 41.1 months, respectively). In this trial, DI-TMZ-induced lymphopenia facilitated homeostatic expansion of pp65 responses after an initial DI-TMZ cycle but later ablated T cell responses when monthly cycles were reintroduced. The benefit of DI-TMZ thus warrants further study in a larger series of patients. Here we describe the initiation of a validation trial, "I-ATTAC: Improved Anti-Tumor Immunotherapy Targeted Against Cytomegalovirus in Patients with Newly-Diagnosed WHO Grade IV Unmethylated Glioma" (IND-16301). This prospective single arm Phase 2 trial of 48 patients will validate our findings that tetanus preconditioning and GM-CSF in conjunction with pp65-DCs extends OS in patients with newly diagnosed, unmethylated GBM. By withholding additional TMZ cycles in this patient population, we hypothesize that CMV immune responses will not be abrogated as previously observed, and that this greater expansion will translate into further prolonged survival (primary objective). A series of secondary objectives will be evaluated for the association between increased DC migration, systemic mediators of tetanus preconditioning, and T cell polyfunctionality with survival.

RBTT-03. A PHASE 1, MULTICENTER, RANDOMIZED, OPEN-LABEL, PERIOPERATIVE STUDY OF AG-120 (IVOSIDENIB) AND AG-881 IN PATIENTS WITH RECURRENT, NONENHANCING, IDH1-MUTANT, LOW-GRADE GLIOMA

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Mutations in isocitrate dehydrogenase (mIDH) are common in lower-grade glioma (LGG; mIDH1, 80%; mIDH2, 4%) and lead to epigenetic and genetic changes that promote oncogenesis via production of the oncometabolite 2-hydroxyglutarate (2-HG). AG-120 (ivosidenib) is a first-in-class oral mIDH1 inhibitor associated with a favorable safety profile in an ongoing phase 1 study in 66 glioma patients. AG-881 is a brain-penetrant oral mIDH1/2 inhibitor with an acceptable safety profile at dose levels <100 mg in an ongoing phase 1 study in 52 glioma patients. In an orthotopic mIDH1-R132H glioma model, ivosidenib and AG-881 suppressed 2-HG by 85% and 98%, respectively. This multicenter, open-label, phase 1 study is designed to measure brain tumor 2-HG concentration and drug exposure at two dose levels each of ivosidenib or AG-881 in patients with mIDH1 LGG undergoing craniotomy (NCT03343197). The study will enroll ~45 adults with recurrent WHO 2016-classified Grade 2 or 3 mIDH1 oligodendroglioma/astrocytoma eligible for resection. Key eligibility criteria include: nonenhancing disease by T2 FLAIR MRI, mIDH1-R132H, and Karnofsky Performance Score 60. Cohort 1 patients will be randomized 2:2:1 to 500 mg QD ivosidenib (n=10), 50 mg QD AG-881 (n=10), or no treatment (n=5). Based on the safety, 2-HG, and pharmacokinetic data from cohort 1, cohort 2 may enroll an additional 20 patients randomized 1:1 to 250 mg BID ivosidenib or 10 mg QD AG-881. Patients randomized to either drug will receive treatment for 4 weeks preoperatively and may continue treatment after surgery. Untreated patients can opt to be randomized to either drug postoperatively. The primary endpoint is 2-HG concentration in surgically resected tumors. Secondary endpoints include safety, tumor and plasma pharmacokinetics, and RANO LGG response. Exploratory endpoints include 2-HG and pharmacokinetics in cerebrospinal fluid, and 2-HG magnetic resonance spectroscopy. This study is currently enrolling in the USA.

RBTT-04. DOES EXERCISE IMPROVE PROGRESSION FREE SURVIVAL AND QUALITY OF LIFE IN PATIENTS WITH GLIOBLASTOMA? A TRIAL IN PROGRESS

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BACKGROUND: Glioblastoma is the most common adult malignant glioma, with poor prognosis and adverse neurological sequelae. Physical activity improves outcomes in patients with other cancers, but has not been evaluated in GBM. This prospective, single-arm intervention trial examines

feasibility and preliminary efficacy of exercise on progression-free survival (PFS), cognition and quality of life (QOL) in newly diagnosed GBM patients. METHOD: Participants are English-speaking GBM patients scheduled for concurrent chemo-radiation at the Princess Margaret Cancer Centre, 18–65 years old, ECOG 2. The 3-month home-based exercise program, prescribed by a physiotherapist, includes aerobic and resistance training, tailored to prior fitness level, current physical status, and individual interests. Assessments of physical and neurocognitive functions, mood, fatigue, sleep, and QOL, occur within 2 weeks of starting chemo-radiation, and approximately 3, 6, 12, and 18 months later, or until tumor progression. Feasibility will be assessed by accrual, retention, and adherence rates. Outcomes include PFS (RANO criteria), change in cognition, physical activity and sleep (actigraphy, self-report questionnaires). Time-to-event outcomes will be estimated (Kaplan-Meier), and mixed modelling will explore individual and disease variables that contribute to outcomes. RESULTS: During the first seven months of recruitment, 51 newly diagnosed GBM patients scheduled to be treated at our institution were screened, 25 met eligibility criteria, and 16 consented. Four participants did not complete the exercise program; 3 withdrew consent, 1 refused concurrent chemoradiation. Two participants died after the intervention and one other progressed. No exercise-related serious adverse events occurred. Updated accrual and feasibility details will be presented at the meeting. DISCUSSION: Exercise appears feasible for GBM patients. Preliminary efficacy in terms of survival, performance status, cognition, sleep, mood, and QOL are under study. Results may guide physical activity recommendations in GBM, lay the groundwork for a larger randomized controlled trial, and generate avenues for translational research.

RBTT-05. TUMOR TREATING FIELDS AND RADIOSURGERY FOR SUPRA- AND/OR INFRATENTORIAL BRAIN METASTASES (1–10) FROM NSCLC IN THE PHASE 3 METIS STUDY

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BACKGROUND: Tumor Treating Fields (TTFields) are non-invasive, loco-regional, anti-mitotic treatment modality comprising low intensity alternating electric fields. TTFields has demonstrated efficacy in non-small cell lung cancer (NSCLC) in in vitro and in vivo models, and in a phase I/II clinical study. TTFields treatment to the brain was safe and extended overall survival in newly-diagnosed glioblastoma. This prospective, multicenter study [NCT02831959] investigated the efficacy, safety and neurocognitive outcomes of TTFields in NSCLC patients with brain metastases (BMs). METHODS: NSCLC patients (N=270) with 1–10 BMs are randomized 1:1 to stereotactic radio surgery (SRS) followed by continuous TTFields ((150 kHz, > 18 hours/day) within 7 days of SRS or supportive care. The TTFields portable device delivers TTFields to the brain using 4 transducer arrays and allows normal daily activities. Patients receive the best standard-of-care for their systemic disease. Patients are followed every two months until second intracranial progression. Patients in the control arm could cross over to TTFields at the time of second intracranial progression. Key inclusion criteria: KPS 70, new diagnosis of 1 inoperable or 210 supra- and/or infratentorial BMs from NSCLC amenable to SRS; KPS 70; and optimal therapy for extracranial disease. Prior WBRT or surgical resection of metastases, a single resectable lesion or recurrent BMs were exclusionary. Primary endpoint was time to 1st intracranial progression. Secondary endpoints included time to neurocognitive failure (HVLT, COWAT and TMT), overall survival, radiological response rate (RANO-BM and RECIST V1.1); quality-of-life; adverse events; time to first/second intracranial progression for patients with 14 and 510 BMs; bi-monthly intracranial progression rate from 212 months; and time to second intracranial and distant progression. The sample size (N=270) was calculated using a log-rank test (Lakatos 1988 and 2002) with 80% power at a two sided alpha of 0.05 to detect a hazard ratio of 0.57.

RBTT-07. NUTMEG: A RANDOMISED PHASE II STUDY OF NIVOLUMAB AND TEMOZOLOMIDE (TMZ) VS TMZ ALONE IN ELDERLY PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA (GBM): TRIAL IN PROGRESS

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BACKGROUND: An increase of mutations as we age is well documented in GBM and in cancer in general. Elderly patients with GBM may have higher mutational burden and may be more likely to respond to immunotherapies. NUTMEG is a randomised Phase II study comparing post radiation NivolUmab and TMZ versus TMZ alone in Elderly patients with newly diagnosed GBM. METHODS: 102 patients will be randomized in a 2:1 allocation to receive short course RT (40Gy/15 daily fractions) and TMZ 75mg/m2) followed by 6 cycles of adjuvant TMZ (150-200mg/m2 days (D) 1–5 q28 days) with Nivolumab (240 mg D1, 15 q28 days for cycles (C) (1–4; 480 mg D1 Q 28 days for C5-6) versus 6 cycles of adjuvant TMZ (150-200mg/m2 D1-5 q28) alone. The study is stratified for ECOG performance status, age (< 70 vs 70), MGMT methylation and extent of resection. An independent safety monitoring committee is overseeing the trial and will review safety data for the first 10 patients treated on the experimental arm (TMZ + Nivolumab). The primary endpoint is Overall Survival (OS). Secondary endpoints include: 6 month Progression Free Survival, adverse events (AEs) and immune AEs, Quality of life, neurological function (NANO Scale), and correlation of modified RANO and iRANO in the experimental arm. Translational research endpoints include correlation of clinical endpoints with mutational burden, comprehensive immune characteristics and novel MRI sequences including pH-weighted MRIs. The expected proportion of patients alive at 24 months is predicted to be 15.7%. A hazard ratio of more than 0.69 in favour of the Nivolumab + TMZ arm will be considered sufficient to warrant further investigation including converting this study into a phase III trial. PROGRESS: At 3 June 2018, 6/18 study sites are open in Australia with 5 patients randomized. ACTRN12617000267358.

RBTT-08. EORTC 1709/CCTG CE.8: A PHASE III TRIAL OF MARIZOMIB IN COMBINATION WITH STANDARD TEMOZOLOMIDE-BASED RADIOCHEMOTHERAPY VERSUS STANDARD TEMOZOLOMIDE-BASED RADIOCHEMOTHERAPY ALONE IN PATIENTS WITH NEWLY DIAGNOSED GLIOBLASTOMA

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BACKGROUND: The standard of care for patients with newly diagnosed glioblastoma includes maximum safe surgery, involved-field radiotherapy (RT), and concomitant and up to six cycles of maintenance temozolomide (TMZ) chemotherapy (TMZ/RT→TMZ). However, the prognosis remains poor and there is a high unmet need to provide new drugs to patients with glioblastoma. Marizomib is a novel, brain-penetrant irreversible pan-proteasome inhibitor that has been successfully assessed in phase I studies in patients with newly diagnosed as well as recurrent glioblastoma. METHODS: EORTC 1709/CCTG CE.8 is a multicenter, randomized, controlled, open label phase III superiority trial. To be eligible, patients need to have histologically confirmed newly diagnosed glioblastoma. A total of 750 patients will be enrolled and randomized 1:1. Stratification factors include institution, age, Karnofsky performance status and extent of surgery. The primary objective of this study is to compare overall survival in patients receiving marizomib in addition to standard treatment (TMZ/RT→TMZ) with patients receiving standard treatment only. The testing strategy is defined to assess this objective in both the intent-to-treat population and the subgroup of patients with tumors harboring an unmethylated O^6-methylguanine-DNA methyltransferase (MGMT) promoter. Secondary endpoints include progression-free survival, safety, neurocognitive function and quality of life. An accompanying translational research program has been set up. The study will be opened at 50 EORTC sites in Europe and done as an intergroup collaboration with the Canadian Cancer Trials Group (CCTG) with 25 sites in Canada and additional sites in the US. Patient enrolment is planned to start in June 2018 and an update on the enrolment status will be provided at the SNO conference. ClinicalTrials.gov Identifier: NCT03345095

RBTT-09. THE EFFICACY OF KETOGENIC DIET WITH CONCOMITANT INTRANASAL PERILLYL ALCOHOL AS A NOVEL STRATEGY FOR THERAPY OF RECURRENT GLIOBLASTOMA

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It has been hypothesized that persistent ketotic hypoglycemia might represent a potential therapeutic strategy against high-grade gliomas. Perillyl alcohol (POH) is a non-toxic, naturally-occurring, hydroxylated monoterpene that exhibits cytotoxicity against TMZ-resistant glioma cells, regardless of MGMT promoter methylation status. This study aimed to evaluate the toxicity and therapeutic efficacy of intranasal POH administered with a ketogenic diet (KD) program for the treatment of patients with recurrent glioblastoma. Thirty-two patients were divided into two groups - KD or standard diet, both associated with intranasal POH (n=17 and n=15, respectively). The nutritional status and anthropometric parameters of patients were measured. Patients that adhered to the KD maintained a strict dietary regimen, while receiving inhalation of POH (55 mg, four times daily) in an uninterrupted administration schedule for three months. Neurological examination and imaging analysis were used to monitor disease progression. Clinical toxicity and overall survival following treatment were correlated with tumor size, topography, extent of peritumoral edema, and frequency of seizures. In the KD patient, strict compliance with the KD was confirmed by measuring the levels of ketone bodies in the urine (9/17 patients) at three times a week. After three months of well tolerated treatment, we observed a *partial response* in 77.8% (7/9 patients), *stable disease* in 11.1% (1/9) and 11.1% (1/9) presented with *progressive disease*. Among the patients assigned to the standard diet (control group), the *partial response* was 25% (2/8 patients), *stable disease* was 25% (2/8), and *progressive disease* was 50% (4/8 patients). The patients assigned to the KD group presented: reduced frequency of seizures; a slight increase in lean muscle mass; reduced serum lipid levels; and decreased low-density lipoprotein cholesterol (LDL-C) levels. Our results are encouraging and suggest that KD associated with intranasal POH may represent a viable option as an adjunct therapy for recurrent GBM

RARE TUMORS

RARE-01. IN VIVO IMMUNOMODULATING EFFECT OF LXR-623 AND ITS SYNERGISM WITH CHECKPOINT BLOCKADE AGAINST CHORDOMAS IN A HUMANIZED MOUSE MODEL

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INTRODUCTION: We previously developed a humanized mouse model of chordomas, which allowed us to investigate the interaction between human chordomas and human immune cells. This platform is particularly useful for studying immunotherapy against rare cancers such as chordomas, where murine equivalents are currently unavailable. We aim to utilize this model to study synergism between PD-1 blockade and LXR-623, a synthetic agonist of liverX receptors, a class of anti-neoplastic agents disrupting cancer cholesterol-metabolism. Our preliminary data suggest that LXR-623 downregulates PD-L1 on chordoma cell lines and reduces immunosuppressive myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment. METHODS: To achieve immune system reconstitution/humanization, 20 NSG-SGM3 mice were engrafted with human fetal thymus and CD34+ stem cells, whose HLA-types were partially-matched with those of the U-CH1 chordoma cell lines. They were divided into the following groups (n=5 for each group): control group (isotype antibodies (Abs) + vehicle), LXR-623 monotherapy group (isotype Abs + LXR-623 100mg/kg, i.p., daily for 4 weeks), anti-PD-1 Abs monotherapy group (anti-human-PD-1-Abs (Clone: J116), 10 mg/kg, i.p., 3 times/week for 4 weeks), and combination group (anti-human-PD-1 Abs + LXR-623). Anti-tumor activities will be monitored via tumor size measurement, flow cytometric analyses of peripheral blood and spleen as well as chordomas, using various Abs including anti-mouse-CD45, anti-human-CD3, anti-human-CD4, anti-human-CD8, anti-human-CD11b, anti-human-CD14, anti-human-CD15, anti-human-CD19, anti-human-CD25, anti-human-CD45, anti-human-CD45RA, anti-human-CD45RO, anti-human-PD-1, and anti-human-FoxP3, multiplex cytokine analyses including IFN-gamma and TGF-beta, and multiplex-immunohistochemistry. We expect to observe the synergistic inhibitory effect of LXR-623 and anti-PD1-Abs against chordomas through PD-L1 down-regulation and reduction in immunosuppressive MDSC levels mediated by LXR-623. We plan to sacrifice the last animal group on August 24th. Due to the lengthy nature of the experiment (humanization and treatment take 13–14 weeks), the data are not fully available at the time of submission but will be available by the late-breaking deadline.

RARE-02. PRIMARY CNS POSTTRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PCNS-PTLD): RECOGNIZING THE ENTITY, MINIMIZING TREATMENT TOXICITY, AND DEVELOPMENT OF SURVEILLANCE TOOLS IN RENAL TRANSPLANT PATIENTS

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PCNS-PTLD is a rare complication of solid organ transplantation (SOT) and is a distinct entity from primary CNS lymphoma (PCNSL). With fewer than 90 cases reported since 1970, there are no treatment standards. Existing data supports combining reduction of immunosuppression (RI), whole-brain radiotherapy, monoclonal antibody therapy, and systemic/IT chemotherapy. Treating kidney SOT PTLD is complicated by reduced renal clearance, contrast-dye restrictions, and nephrotoxicity. Treatment complications and allograft rejection cause early morbidity and mortality. Rapid identification of serum/CSF supporting diagnostic markers such as active EBV replication, elevated CSF protein, and positive cytology/flow cytometry is critical. We report three cases of B/T-cell PTLD in renal-SOT patients 1–27 years on immunosuppression (steroids, mycophenolate, tacrolimus/sirolimus) who presented with focal neurologic deficits and multifocal MRI lesions. After confirming CNS-isolated disease patients were treated with partial-RI, dexamethasone, and induction with rituximab weekly cycles. Patients had objective responses and underwent consolidative rituximab therapy q21-days with restaging. Residual enhancing lesions were treated by stereotactic radiosurgery and one year of temozolomide chemotherapy days 1–5 on a 28-day cycle. We monitored serum LD, drug levels, and serum EBV as indicators for progression/recurrence. Patients achieved complete responses and mOS has not been met. Long-term survival in PCNS-PTLD is one distinguishing feature from PCNSL. With multiple treatment obstacles in renal-SOT PT early recognition and minimally toxic regimens improve patient outcomes. This data challenges traditional paradigms in diagnosis and treatment of PTLD, specifically EBV+ disease occurring >6 years post SOT. We propose that the specialized CNS immune microenvironment permits EBV driving of neoplastic progression, which is exquisitely sensitive to B-cell targeting concurrent with partial restoration of host immunity. Furthermore, targeted radiotherapy, temozolomide, and partial immunosuppression maintains patient quality-of-life and reduce risk for allograft rejection.

RARE-03. CHARACTERISTICS OF PATIENTS WITH NEUROCUTANEOUS MELANOSIS: THE MSK EXPERIENCE FROM 2003–2018

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BACKGROUND: The incidence of large congenital melanocytic nevi (LCMN) is 1/20,000 with 25% of children diagnosed with neurocutaneous melanocytosis (NCM), characterized by excessive proliferation of melanocytes in the leptomeninges and brain parenchyma. Specifically the disorder represents abnormal migration of melanocyte precursors due to abnormal melanin-producing genes in primitive leptomeningeal cells. While NCM can transform to melanoma, the molecular drivers involved in NCM related melanoma are different from the drivers in patients with non-NCM related melanoma. Children who become symptomatic from NCM may have a poor prognosis overall with 25–60% developing melanoma. While melanoma typically harbors mutations in the BRAF gene, NCM has a distinct molecular signature comprised primarily of mutations in the NRAS gene. METHODS: We reviewed our institutional database from 2003–2018 and identified 47 patients with large congenital melanocytic nevi (LCMN) and NCM. Fifteen were female and 16/47 died. Five had molecular testing which revealed an NRAS mutation in the affected tissues. Four patients who developed melanoma, received targeted therapy including off label trametinib, nivolumab and/or ipilumumab. While none of these patient were longterm survivors, one patient lived for 12 months after receiving radiation therapy, nivolumab and ipilumumab. One patient with a VP shunt developed intraperitoneal melanoma, malignant ascites and subsequently died. CONCLUSIONS: While NCM which transforms to melanoma is typically fatal, new targeted therapies may offer prolonged survival. Further efforts to identify which patients are high risk are needed in an attempt to offer earlier treatment.

RARE-04. PDL1 LOSS OF EXPRESSION IN A METASTATIC CARCINOMA OF UNKNOWN PRIMARY WITH HEPATOID FEATURES TREATED WITH NIVOLUMAB

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INTRODUCTION: The anti-PD-1 monoclonal antibody nivolumab, used in metastatic melanoma, renal carcinoma, and advanced non-small cell lung cancer, may be used off-label in some PD-L1 positivite tumors. We report the first use (to our knowledge) of nivolumab in metastatic carcinoma of unknown primary with hepatoid features, with subsequent loss of PD-L1 expression on the tumor. CASE PRESENTATION: A 62-year old man complained of worsening back and right thigh pain. X-rays revealed an L3 compression fracture, which was managed non-operatively. He developed worsening right hip pain. Imaging revealed a right iliac mass, associated pathologic fracture, and multiple osteoblastic and osteolytic metastases. RESULTS: Iliac mass biopsy showed cords and nests of malignant epithelial cells with abundant eosinophilic cytoplasm, irregular nuclear contours, and prominent nucleoli. HepPar, Cam5.2, and PAX-8 (focal) immunohistochemical stains were positive, suggesting hepatic carcinoma. PD-L1 positivity was high (> 25% but < 50%). Genomic sequencing showed NF2, MLL2, and SETD2 mutations. Workup showed no hepatic involvement, so stage IV hepatoid carcinoma of unknown primary was diagnosed. TREATMENT: Several bony lesions were radiated. Nivolumab (240 mg every 2 weeks) and denosumab (monthly) were started. PROGRESSION: Seven months later he developed left ptosis and proptosis from a sellar/left cavernous sinus mass. Histology was similar to the previous iliac biopsy, but with more aggressive features (mitoses, atypical nuclei). Cam5.2 and synaptophysin immunohistochemical stains were positive; PD-L1 expression was <1%. Genomic sequencing showed PTEN, ERRFI1, CREBBP, EP300, LRP1B, MSH2, RB1 and P53 mutations, and increased mutational burden status (intermediate from low). DISCUSSION: PD-L1 expression may vary over time. Tumors can express PD-L1 constitutively or in response to IFNg from infiltrating lymphocytes. PD-L1 expression may decrease after IFNg levels decrease. Complete loss of PD-L1 has not, to our knowledge, been reported. CONCLUSION Loss of PD-L1 expression may represent a means by which tumors resist immunotherapy.

RARE-05. TUMOR PROFILING REVEALS EPITHELIAL-TO-MESENCHYMAL TRANSITION (EMT) AND ENHANCED IMMUNE SUPPRESSION IN GLIOSARCOMAS RELATIVE TO GLIOBLASTOMA

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BACKGROUND: Gliosarcoma (GS) accounts for approximately 2% of WHO grade 4 gliomas and have a worse prognosis relative to glioblastoma (GBM). Epithelial-to-mesenchymal transition (EMT) is well recognized in a variety of tumors of ectodermal lineage. MATERIALS AND METHODS: Patients diagnosed with either a GS (n = 44) or GBM (n = 1449) underwent comprehensive molecular profiling including immunohistochemistry (IHC) and sequencing (DNA, RNA, pyrosequencing and fragment analysis). Statistical significance using Fisher's exact test was set at $p < 0.1$ as this is exploratory work; no correction was made for multiple comparisons. RESULTS: The following were identified as enriched in GS based on the odds ratios (OR): CALR (copy number variation, CNV; OR= infinite; p = 0.032), LYL1 (CNV; OR=30; p=0.064); NTRK1 (fusion, RNAseq; OR= 30; p=0.063), IDH2 (mutation; OR=28; p=0.068), PTPN11 (mutation; OR=3; p=0.08), NF1 (mutation; OR=2.3; p=0.02; PD-L1 (IHC; OR=2.2; p=0.002); PD-1 (IHC; OR=1.6; p=0.0078). In contrast, the following were more common in GBM: EGFRvIII (fusion, RNAseq; OR=0.28; p=0.031); EGFRvIII (fragment analysis; OR=0.2; p=0.062); EGFR (CNV; OR=0.2; p=0.00083; IDH1 (mutation; OR=0; p=0.045) and EGFR (mutation; OS=0; p=0.012). Alterations with p>=0.1 are not reported here. CONCLUSIONS: Our study identified enriched markers in GS that have been associated with EMT in other tumor types. These findings are hypothesis generating and suggest that GS may be an example of EMT in GBM. The gene amplification in CALR and LYL1 has not been previously described. Higher levels of PD-1 and PD-L1 expression suggest that these may be targets of particular therapeutic importance in GS relative to GBM.

RARE-07. THE EFFECT OF SELUMETINIB ON SPINAL NEUROFIBROMAS IN PATIENTS WITH NF1

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BACKGROUND: Spinal neurofibromas (SNF) in neurofibromatosis type 1 (NF1) can cause progressive cord compression with associated morbidity. A phase 1 study of the MEK inhibitor Selumetinib in patients with plexiform neurofibromas (PN) demonstrated PN shrinkage and improvement clinical symptoms. Here we describe the effect of selumetinib on SNF burden. METHODS: Participants of the ongoing phase 2 pediatric (NCT01362803) and adult (NCT02407405) selumetinib studies for NF1 PN, who underwent serial spine MRI exams were examined. The effect of selumetinib on the size of SNF, shape of the spinal canal, spinal cord, and cerebrospinal fluid (CSF) space were evaluated at baseline and during treatment by linear measurements. RESULTS: Nineteen patients (14 male), median age 15.5 years (range, 6–60) had documented SNF and were included in the analysis. Six patients had prior spinal decompression surgery and all had SNF extending into the central canal at one or more levels (14 cervical, 2 thoracic, 12 lumbosacral). Deformation of the spinal cord cross-section was observed in 17 patients. Sixteen patients completed at least 12 cycles of treatment, and 3 received 8 cycles. Tumors remained stable in 5 patients, improvement was observed in 14 patients (9 children, 5 adults) and no SNFs exhibited progression during treatment. Most improvements were observed after 4 cycles and were maintained. The most notable change was expansion of the CSF space (due to tumor shrinkage), with CSF signal becoming circumferential. Analysis of clinical correlations is ongoing. CONCLUSIONS: This is the first study assessing selumetinib effect on SNF. In 14 of 19 patients with SNF, we observed prolonged improvement in SNF attributes. Our findings indicate selumetinib may prevent worsening of cord compression, and reduce surgical interventions. The role of MEK inhibitor therapy for the treatment of SNF needs to be prospectively evaluated along with functional measures and patient reported outcomes.

RARE-08. GRADING CONSIDERATIONS FOR MENINGEAL SOLITARY FIBROUS TUMOR/HEMANGIOPERICYTOMA

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Meningeal solitary fibrous tumor (SFT)/hemangiopericytoma (HPC) is a rare tumor with propensity for recurrence and metastasis. We compared the WHO 2016 CNS tumor classification (CNS-G), a 3-tier system based on histopathologic phenotype and mitotic count, to the WHO 2013 Soft Tissue classification (ST-G), a 2-tier system based on mitotic count alone, in a cohort of 133 patients (59 female, 74 male; mean age 54 years [range 20–87]) with meningeal SFT/HPC. Tumors were pathologically confirmed through review of the first tumor resection (n=97), local recurrence (n=35) or distant metastasis (n=1). STAT6 immunostain showed nuclear expression in 132 cases. *NAB2-STAT6* fusion was detected in 99 (of 111) successfully tested tumors (89%) including the lone STAT6 immunonegative tumor. Tumors were classified as grade 1 (n=43), 2(n=41) or 3 (n=49) using the CNS-G, and SFT (n=84) or malignant SFT (n=49) by using the ST-G. Necrosis was present in 16 cases (12%). On followup, 42 patients had at least 1 subsequent recurrence, including 8 with metastases. 29 patients died.

On univariate analysis necrosis (p=0.0018) and CNS-G or ST-G (p-value respectively 0.014, 0.0041) were significantly associated with recurrence-free (RFS) but not overall survival (OS). *NAB2-STAT6* fusion type was not associated with RFS or OS. Ten-year RFS was 61, 58 and 34% for CNS-G 1,2 and 3 versus 59 and 34% for ST-G SFT and malignant SFT, respectively. Ten-year RFS was 0% and 56% for tumors with and without necrosis. Our data suggest that SFT/HPC are better stratified using a two-tiered grading scheme. On multivariate analysis, necrosis was an independent predictor of RFS (HR 2.9, p=0.016) while ST-G was not quite significant (HR=1.9, p=0.062), suggesting necrosis should be reintroduced among SFT/HPC grading criteria.

RARE-09. EFFICACY AND SAFETY OF DABRAFENIB + TRAMETINIB IN PATIENTS WITH RECURRENT/REFRACTORY BRAF V600E–MUTATED HIGH-GRADE GLIOMA (HGG)

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BACKGROUND: Current treatment outcomes for patients with recurrent HGG are poor, with median progression-free survival (PFS) and overall survival (OS) of 2.5 and 7.5 months, respectively. Dabrafenib (BRAF inhibitor) + trametinib (MEK inhibitor) resulted in an intracranial overall response rate (ORR) of 58% in *BRAF* V600–mutated melanoma brain metastases. Dabrafenib + trametinib was evaluated as treatment for patients with *BRAF* V600E–mutated HGG. METHODS: In this phase 2, open-label trial (NCT02034110), patients with *BRAF* V600E mutations in 9 rare tumor types, including HGG, received continuous dabrafenib (150 mg BID) + trametinib (2 mg QD) until unacceptable toxicity, disease progression, or death. For the HGG cohort, eligible patients had histologically confirmed recurrent or progressive WHO grade 3 or 4 glioma and had prior treatment with radiotherapy and first-line chemotherapy or concurrent chemoradiation therapy. The primary endpoint was investigator-assessed ORR by RANO criteria. Secondary endpoints included duration of response (DOR), PFS, OS, and safety. RESULTS: Thirty-seven patients with HGG had enrolled at data cutoff (3 January 2018). Median age was 42 years, 31 of 37 patients were evaluable for response. Investigator-assessed confirmed ORR was 26% (8/31; 95% CI, 12%-45%), including 1 complete response (CR). Six of 8 responses were ongoing at data cutoff. Five of 8 responding patients had a DOR of ≥ 12 months. Median PFS was 1.9 months (95% CI, 1.7–18.5). Median OS was 11.7 months (95% CI, 6.4-not reached). Adverse events (AEs) in patients with HGG included fatigue (35%), headache (30%), and rash (24%). Grade 3/4 AEs included neutropenia (8%) and fatigue (5%). Biomarker analyses are ongoing and will be presented. CONCLUSIONS: Dabrafenib + trametinib demonstrated promising efficacy in patients with *BRAF* V600E-mutated recurrent/refractory HGG, as 1 patient had a CR, and most responders had a prolonged DOR.

RARE-10. INITIAL EXPERIENCE IN EPENDYMOMA WITH INVESTIGATIONAL CANCER-TARGETING BXQ-350 SapC-DOPS NANOVESICLES: A RARE TUMOR CASE STUDY

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BACKGROUND: Ependymomas are rare primary nervous system tumors accounting for about 3% of adult brain tumors in the United States. The current standard of care includes surgical resection and radiation therapy, with more than half experiencing relapse and poor outcomes. Saposin C and dioleylphosphatidylserine (SapC-DOPS) nanovesicles are a novel investigational treatment agent that can cross the blood-brain barrier and selectively target externalized phosphatidylserine that is expressed on tumor cell surfaces. METHODS: We examined a rare complex ependymoma case enrolled in the ongoing Phase 1b study of SapC-DOPS cancer-targeting agent BXQ-350 (NCT02859857). The patient received cycle 1 (BXQ-350 2.4 mg/kg IV infusion at Day 1–5, 8, 10, 12, 15, 22) and 3 additional cycles (1x28 days), and was followed until death for safety, response, RANO, and ECOG. RESULTS: A 67-year old white male with recurrent Grade III anaplastic ependymoma diagnosed 3 years before enrollment and with a history of surgical intervention exhibited measurable extra-axial (6.4x3.2 cm) and inferolateral intracranial components on MRI, accompanied by palpable skull mass. At baseline, the lesion was 6.4 x 3.2 cm with stable disease per RANO and ECOG of 1. After 2 cycles, minor decrease in size of intracranial enhancing components was reported (overall stable disease per RANO). The patient received 4 total cycles of BQX-350 without related adverse events or toxicities, and cycle 5 was withheld due to volume progression on MRI. He died 6 months post-enrollment. Post-mortem histology and gross anatomy showed extensive tumor necrosis with chondroid differentiation and gross signs of metastatic disease in the lungs, thoracic, and lumbar spine on microscopy. CONCLUSIONS: While further study of cancer-targeting SapC-DOPS nanovesicle efficacy in adult patients with ependymoma is needed, good tolerability in rare aggressive tumors was observed. The role of extensive necrosis and possibly treatment-related intracranial mass reduction supports additional investigations in this indication.

RARE-11. EFFICACY AND SAFETY OF DABRAFENIB + TRAMETINIB IN PATIENTS WITH RECURRENT/REFRACTORY BRAF V600E–MUTATED LOW-GRADE GLIOMA (LGG)

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BACKGROUND: Approximately 9%-18% of LGGs possess *BRAF* V600E mutations. Combined BRAF and MEK inhibition is efficacious in *BRAF* V600–mutated melanoma, lung cancer, and anaplastic thyroid cancer. Dabrafenib (BRAF inhibitor) + trametinib (MEK inhibitor) was evaluated as treatment for patients with recurrent/refractory *BRAF* V600E–mutated LGG. METHODS: In this phase 2, open-label trial (NCT02034110), patients with *BRAF* V600E mutations in 9 rare tumor types, including LGG, received continuous dabrafenib (150 mg BID) + trametinib (2 mg QD) until unacceptable toxicity, disease progression, or death. For the LGG cohort, eligible patients had histologically confirmed recurrent or progressive WHO grade 1 or 2 glioma that was refractory to standard-of-care therapies. The primary endpoint was investigator-assessed overall response rate (ORR) by RANO criteria. Secondary endpoints included duration of response (DOR), progression-free survival (PFS), overall survival (OS), and safety. RESULTS: Nine patients with LGG had enrolled at data cutoff (3 January 2018). Eight of 9 patients were evaluable for response. Median age was 33 years. Eight of 9 patients had received prior surgery. Investigator-assessed confirmed ORR was 50% (4/8; 95% CI, 16%-84%), with 3 of 4 responses ongoing at data cutoff. Two of 4 patients had a DOR of ≥ 18 months. The PFS and OS Kaplan-Meier estimates at 18 months were 50% (95% CI, 15%-78%) and 86% (95% CI, 33%-98%), respectively. Adverse events (AEs) in patients with LGG included fatigue (67%), headache (67%), arthralgia, nausea, and pyrexia (56% each). Grade 3/4 AEs included fatigue (22%), arthralgia, headache, and diarrhea (11% each). Biomarker analyses are ongoing and will be presented. CONCLUSIONS: Dabrafenib + trametinib demonstrated promising efficacy in patients with recurrent/refractory *BRAF* V600E-mutated LGG, with manageable AEs and no new safety signals

RARE-13. CHARACTERIZATION OF ADULT MEDULLOBLASTOMA PATIENTS AT RECURRENCE: RETROSPECTIVE REVIEW OF THE MD ANDERSON CANCER CENTER EXPERIENCE

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BACKGROUND: Adult medulloblastoma (MB) is rare, accounting for 150–300 new cases in the US annually. Given the low incidence, prospective studies are challenging, and treatment guidelines and recurrence characteristic are derived from pediatrics. However, adult MB is a distinct entity, and further studies are warranted. Surgery followed by radiation and chemotherapy are accepted treatment modalities, but there is limited information on characteristics and outcomes of adult MB at recurrence. METHODS: 136 adult MB patients (≥18 years at diagnosis) were identified at MD Anderson from January 1978 to April 2017. Median progression-free survival (PFS) and overall survival (mOS) were estimated using the Kaplan-Meier method. RESULTS: Median age 28 (18–63); 53.7% standard risk (SR) (minimal residual disease and no metastasis) and 32.4% high risk (HR) (indeterminate in 19). Median PFS and mOS for the entire cohort were 98 and 133 months, respectively. No residual tumor after initial surgery and use of adjuvant chemotherapy were associated with improved survival, but M status was not. 44.9% had developed recurrence at median follow-up of 59.5 months (34.4% were SR and 42.6% were HR); median time to 1st recurrence was 34 months; mOS after first recurrence was 23 months. Local recurrence was associated with improved survival compared to distant recurrence. CONCLUSION: To our knowledge, this is the largest series of adult MB from a single institution. Extent of surgery was associated with improved survival, which is an established positive prognostic indicator in pediatric MB but has been controversial in adults. Adjuvant chemotherapy was associated with improved survival in patients without residual tumor after first surgery. Late recurrences were more common than in the pediatric population, highlighting the need for long term follow-up. Adult MB patients have a poor mOS of 23 months after recurrence, and optimizing treatments at recurrence is an unmet need.

RARE-16. CLINICAL AND HISTOPATHOLOGICAL CHARACTERISTICS OF YOUNG ADULTS WITH GLIOBLASTOMA AT DIAGNOSIS

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BACKGROUND: Glioblastoma (GBM) occurs commonly in 6th to 7th decades of life. GBM in young adults (age 18–39 yrs) is rare and the implications of this diagnosis during young adulthood has different considerations than in their older counterparts. We planned to identify young adults with GBM and to evaluate the clinical and histopathological factors in this rare group. METHODS: We queried retrospectively an IRB-approved database registry from 12/2004- 10/2016. We included GBM patients by these factors: 1. Age at diagnosis as 18–39 yrs, 2. Confirmed GBM pathology at initial diagnosis, and 3. Usable information on time to first progression. We excluded patients with an initial diagnosis of WHO grades II-III glioma. We obtained available clinical and histopathological information. We estimated progression-free survival (PFS) and overall survival (OS) using Kaplan-Meier methods. RESULTS: We found 184 young adult patients that met our criteria for inclusion in this evaluation. Median age was 33 yrs with a majority being males (n=109, 59.2%). The majority (89.1%) had surgical resection at time of diagnosis (GTR, n=106 and STR, n=58). Median OS was 41.6 mos (95% CI: 31.2, 51.6), with a 5-year OS of 38.9% (95% CI: 31.2%, 46.5%). Median PFS was 17.2 mos (95% CI: 14.1, 21). Recurrence occurred in 148 patients (80%) and 25% (n=37) were enrolled in clinical trials after 1st recurrence. While most had pathology described as GBM, there were 50 variants (27.2%) including giant cell (n=20), small cell (n=15), oligodendroglial (n=10), PNET (n=4), and rhabdoid (n=1). Known IDH1 status was 29 mutant (15.8%) and 51 wild type (27.7%) CONCLUSIONS: Diagnosis of GBM in young adults associated with a longer OS. Surgical resection at diagnosis and participation in clinical trials are common. Histopathology for this group can be heterogeneous. Providers should tailor treatment and survivorship planning uniquely to the young adult GBM population.

RARE-17. LENALIDOMIDE AS TREATMENT FOR RELAPSED OR REFRACTORY PRIMARY CNS LYMPHOMA: A SINGLE INSTITUTIONAL EXPERIENCE

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BACKGROUND: Primary CNS Lymphoma (PCNSL) is an aggressive form of diffuse large B-cell lymphoma (DLBCL). Lenalidomide is an immunomodulatory agent which has shown activity in relapsed or refractory CNS and non-CNS DLBCL. The ideal dosing, timing as consolidation therapy, and role in maintenance therapy remain unclear. METHODS: We describe 3 elderly patients with R/R PCNSL who were treated with lenalidomide. All patients had a pathological diagnosis of PCNSL, immunocompetent status, failed at least 2 previous therapies including methotrexate and rituximab. One patient had also failed autologous stem cell transplant. Lenalidomide was administered orally, 25mg/day on days 1–21 of a 28-day cycle. RESULTS: Median age was 68 (range 65–71). Median KPS was 70 (range 60–70). Patient 1 achieved complete response (CR) after cycle 4, 2 additional maintenance cycles (20mg; reduced for Grade 3–4 Thrombocytopenia), and is in remission after 11 months. Patient 2 achieved partial response (PR) after cycle 1, CR after cycle 8, and 2 additional maintenance cycles; dose reduced at cycle 6 (20mg) for Grade 3–4 Neutropenia, and is in remission after 14 months. Patient 3 achieved PR after cycle 1, mixed response after cycle 2, and died 15 weeks after start of treatment; of note, patient was also on rituximab. Molecular and immunologic correlates are pending and will be presented with the abstract. CONCLUSIONS: This case series shows lenalidomide has single agent activity in heavily pre-treated R/R PCNSL. All the patients had clinical and radiographic response to treatment. Hematologic toxicities occurred at higher dose, however even at reduced dose, lenalidomide was effective in maintaining remission. In elderly patients, using lenalidomide at relapse and deferring neurotoxic treatment options may have a role in preserving good performance status. Further prospective studies are warranted to determine the efficacy and optimal dose of lenalidomide in patients with newly diagnosed and R/R PCNSL.

RARE-18. OSTEOSARCOMA AND MEDULLOBLASTOMA IN A LI-FRAUMENI PATIENT

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Li-Fraumeni patients have germline mutations in the TP53 gene resulting in increased risk of developing one or more malignant tumors. In children, osteosarcomas and rhabdomyosarcomas are the most common tumors outside the central nervous system (CNS). Primary CNS tumors in these patients include glioblastomas, choroid plexus carcinomas and medulloblastomas. We present an unusual case of a 15 year old boy who had a left tibial osteosarcoma resected 4 years ago, then treated with chemotherapy, without any recurrence or metastatic disease. His two younger male siblings both have the same mutation, and one sibling has already developed an osteosarcoma. Interestingly, the mutation is present in the mother, who has no neoplasm to date. Annual radiological studies of the CNS were normal until February 2017; but it was 13 months until the next scan, which revealed a large tumor in the lateral cerebellum. The patient was asymptomatic and a surgical resection was performed. The operative report described a well-circumscribed mass in the lateral cerebellum with little parenchymal involvement bringing up the possibility of metastatic disease. Neuropathological evaluation showed a densely cellular tumor with large and markedly pleomorphic nuclei as well as numerous mitoses. No cerebellar tissue was seen. Immunohistochemical (IHC) studies were complicated by the widely overlapping antigenicity of osteosarcomas and medulloblastomas. Next Generation Sequencing found abnormalities in SMO, DDX3X, TP53, and MYCN amplification, which are associated with SHH-activated medulloblastomas. Both GAB1 IHC and FISH studies for YAP1 were positive. In summary we report a rare case of an SHH-activated medulloblastoma, large cell/anaplastic type in a 15 year-old boy with a germline TP53 mutation and history of resected osteosarcoma.

RARE-19. CHEMOTHERAPY FOR SPINAL GLIOMAS IN ADULTS: A RETROSPECTIVE STUDY

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BACKGROUND: Chemotherapy is a treatment option in patients diagnosed with anaplastic gliomas or glioblastomas of the spinal cord, or with recurrent lower World Health Organization (WHO) grade spinal gliomas that are no longer amenable to local treatment. The low incidence of spinal cord gliomas, particularly in adults, limits the ability to perform clinical trials. The role of chemotherapy in these tumors has remained unclear. METHODS: We performed a retrospective study of 21 patients diagnosed with spinal gliomas who were treated with chemotherapy at any time during the disease course. Benefit from chemotherapy was estimated by applying Response assessment in neuro-oncology criteria. RESULTS: Patients were diagnosed with myxopapillary ependymoma (N=3), anaplastic ependymoma (N=5), pilocytic astrocytoma (N=4), astrocytoma (N=2), anaplastic astrocytoma (N=3) or glioblastoma (N=4). Median follow-up from start of chemotherapy was 59 months. Nine of 12 patients with known MGMT status had tumors with MGMT promoter methylation. Eight patients had chemotherapy as part of first-line treatment: patients with glioblastoma received temozolomide (TMZ) (N=3) or vincristine/etoposide/cisplatin/ifosfamide (N=1); patients with astrocytoma (N=1), with anaplastic astrocytoma (N=2) and with anaplastic ependymoma (N=1) received TMZ. All other patients had chemotherapy at progression which included alkylating agents (N=9), carboplatin-based chemotherapy (N=2), hydroxycarbamid (N=1), or ifosfamide/etoposide/doxorubicin (N=1). Response rates were as follows: myxopapillary ependymoma 3 stable diseases; anaplastic ependymoma 1 partial response (N=2 data missing); pilocytic astrocytoma 1 stable disease; astrocytoma 1 stable disease, anaplastic astrocytoma 2 stable diseases (N=2 data missing); glioblastoma 1 complete response and 1 stable disease. There was no indication for a favorable prognostic role of MGMT promoter methylation. CONCLUSIONS: Spinal cord gliomas represent a heterogeneous group of tumors. Survival outcomes in response to chemotherapy in adult spinal glioma patients vary substantially, but individual patients appear to derive benefit from chemotherapy.

RARE-20. BRAF MUTATIONS IN PEDIATRIC GANGLIOGLIOMAS AND THE CLINICAL SIGNIFICANCE AN MD ANDERSON CANCER CENTER EXPERIENCE

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INTRODUCTION: Ganglioglioma (GG) is a rare mixed glioneuronal neoplasm accounting for 0.5–5% of all pediatric central nervous system tumors. BRAF V600E mutation has been previously reported in GG but its incidence and impact remain unknown. This retrospective study evaluates the incidence of BRAF mutation in GG and its prognostic implication. PATIENTS AND METHODS: Retrospective review of 55 patients under the age of 21 years diagnosed with GG at our institution from 1992 to 2012 was performed. Patient demographics, clinical history, radiological features, treatment data and molecular analysis data were collected and analyzed. Molecular testing using next generation gene sequencing and /or immunohistochemistry (IHC) to identify BRAFV600E mutation was performed in available tumor specimens. Kaplan-Meier survival and Cox-regression analyses were performed to assess the overall survival (OS) and progression-free survival (PFS). RESULTS: 55 patients with GG were identified; tumor tissue was available for 21 patients for molecular analysis. Two specimens were inadequate for analysis, 19 patients specimens were tested for BRAF mutation, 3 using sequencing and 16 using IHC. BRAF V600E was detected in nine patients specimens (47%). BRAF mutation was equally distributed among both genders. No association was found with tumor location, size, metastatic status or imaging characteristics. There was no difference in outcome between patients with BRAF mutated tumors versus non mutated tumors, with the 10 years PFS and OS 77.8% versus 51.4% and 88.9% versus 100% (p=0.26, p= 0.29), respectively. Unfortunately, the study number is small to draw statistical conclusion. CONCLUSION: We present one of the largest retrospective studies in pediatric GG. Though limited sample numbers were probed for BRAF mutation in our study, our data suggests similar incidence as reported in the literature. Prospective studies should continue to evaluate this molecular event, and newer generation of BRAF inhibitors should be profiled which could improve clinical outcome.

RARE-21. A DESCRIPTIVE REPORT OF PATIENTS WITH RARE CENTRAL NERVOUS SYSTEM (CNS) CANCERS ON AN NCI-CONNECT CANCER MOONSHOT IMMUNE CHECKPOINT INHIBITOR TRIAL

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BACKGROUND: There is increasing interest in the use of immunotherapy in rare CNS tumors, but limited experiences in the clinical evaluation of imaging findings and treatment toxicity in these patients. A phase II clinical trial of Nivolumab, a PD-1 inhibitor, in eleven rare CNS tumors was developed by NCI-Connect program. The purpose of this report is to describe both patient-reported outcome (PRO) and standardized assessment of these patients treatment toxicities and correlate with imaging findings. METHODS: Patients were evaluated using standardized CTCAE (version 4.0) for toxicity grading and M.D. Anderson Symptom Inventory-Brain/Spine Tumor Module (MDASI-BT/SP) for PRO scoring. The item change scores (>1 point) in individual symptoms, overall symptom burden, and interference from symptoms were calculated at the time of imaging changes and classified as improved, stable or worsened. RESULTS: Of thirteen patients, male (n=10), median age 43 (24–74), brain location (n=10), spine location (n=4), the most common diagnosis was Ependymoma (n=4). An average of 4 cycles of nivolumab was received, with three grade 3 or higher adverse events: one grade 3 colitis and two grade 3 lymphopenia. Radiographic disease progression was declared in all, confirmed by pathological diagnosis in 8/13. Per MDASI-BT/SP, mean symptom severity was stable in all cases; interference score was worse in 3/8 cases. Significant worsening in disease-related symptoms included walking (50%) and pain (25%). Autonomic function worsened in 2/3 spine patients; concentration/memory and seizures worsened in 60% and 20% respectively in brain tumor patients. CONCLUSION: Nivolumab was well tolerated with no CNS specific adverse events. MRI changes were not associated with change in symptom burden, but was associated with significant worsening in patient-reported interference, specifically in walking, pain, seizures, cognitive or autonomic symptoms. Evaluation of cardinal symptoms associated with progression on immunotherapy may be meaningful diagnostic adjuncts in patients with rare CNS tumors.

RARE-22. FREQUENT HIGH TUMOR MUTATIONAL BURDEN (TMB) AND PD-L1 EXPRESSION IN PRIMARY CNS LYMPHOMA (PCNSL)

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BACKGROUND: PD-1 and PD-L1 expression has not been well-described in PCNSL. In one report of 20 PCNSL tumors, PD-1 and PD-L1 expression on tumor cells (TC), tumor-infiltrating lymphocytes (TIL), or tumor-associated macrophages (TAM) was seen in 90% (Clin Neuropathol 2014;PMID:24359606). Another report of 4 patients with relapsed PCNSL showed clinical and radiographic responses to PD-1 blockade (Blood 2017;PMID:28356247). METHODS: Samples from 36 subjects with large B-cell PCNSL submitted to Caris Life Sciences from 20132018 were tested by NextGen Sequencing (NGS, 592-gene panel [n=30] or 45-gene panel [n=6]) for mutations and gene amplification. RNA sequencing (Archer FusionPlex) was used to detect gene fusions. TMB was calculated using somatic nonsynonymous missense mutation, and microsatellite instability (MSI) was tested by NGS. PD-L1 IHC (SP142) was tested on tumor cells and PD-1 was determined on TIL. RESULTS: High PD-L1 expression (>5% staining) was seen in 14 cases (39%), and low

expression (15% staining) was noted in 11 cases (31%). Another 11 cases (30%) showed absent PD-L1 expression. PD-1 expression (>1 cell/high-power field) was seen in 10/12 tumors (83%) without correlation with PD-L1. TMB of 5 mutations per megabase (mt/MB) was seen in all 30 tumors tested with the 592-gene panel, with 27% (n=8) exhibiting high TMB (17 mt/MB). No samples had high level MSI. Overall, 18/30 tumors (60%) had high PD-L1 or high TMB. Mutations in 25 genes were seen; the most frequent were MYD88 (26/30, 87%, all L265P), PIM1 (16/30, 60%), and CD79B (15/30, 50%, all Y196). Among 7 cases tested with RNA-sequencing, one recently reported ETV6-IGH fusion was found (Neuro-Oncology 2018;PMID:29432597). CONCLUSIONS: We found high TMB and PD-L1 expression in PCNSL with no correlation. Nearly two thirds of PCNSL tumors harbor favorable biomarker profiles with anticipated responsiveness to checkpoint inhibitors. Further studies are needed to validate initial results.

RARE-23. PRIMARY INTRA-AXIAL CENTRAL NERVOUS SYSTEM INFLAMMATORY MYOFIBROBLASTIC TUMOR, ALK NEGATIVE: A RARE ENTITY

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Inflammatory myofibroblastic tumor (IMT) is a rare spindle cell neoplasm with admixed inflammatory cells which occurs mainly in the abdomen or thoracic cavity of children or young adults. Primary CNS IMT is exceedingly rare with roughly 100 reported cases in the world literature, most of which are extra-axial and occur as a dural-based mass. Herein, we describe a rare case of intra-axial primary CNS IMT. A 24 year-old healthy female presented to the ER after falling and striking her head. A CT scan revealed an acute intraparenchymal hemorrhage, as well as a mass within the right frontoparietal lobe. Subsequent MRI was performed further characterizing the lesion as a 3.0 cm intra-axial tumor. Craniotomy was performed displaying a circumscribed neoplasm with relatively bland spindle cells arranged in fascicles with an admixed lymphoplasmacytic infiltrate. Mitotic activity was present but limited. Immunohistochemistry (IHC) was positive for TLE1 and vimentin but negative for GFAP, ALK, SMA, MUC4, KIT and β-catenin. Additional molecular testing by FISH for *ALK* (2p23) rearrangement was negative. We report a rare case of intra-axial primary CNS ALK Negative IMT. Approximately half of all IMTs harbor a clonal translocation that activates the anaplastic lymphoma kinase (ALK)-receptor tyrosine kinase at the 2p23 locus. As a result, *ALK* is overexpressed and can be detected by IHC or via molecular diagnostics (FISH, RNA sequencing or RT-PCR). Since this case was processed, additional novel anomalies involving rearrangements in *ROS1*, *RET*, *ETV6* and/or *NTRK3* genes have been described and could lead to promising therapeutic targets in the future.

RARE-24. OBJECTIVE RESPONSE AND CLINICAL BENEFIT IN RECURRENT EPENDYMOMA IN ADULTS: FINAL REPORT OF CERN 08-02: A PHASE II STUDY OF DOSE-DENSE TEMOZOLOMIDE AND LAPATINIB

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BACKGROUND: Ependymoma is a rare tumor for which the role of chemotherapy has not been established either for newly diagnosed or recurrent disease. We report on the first prospective adult clinical trial of chemotherapy for recurrent ependymoma. METHODS: Adult (> 18 years) were treated with dose-dense temozolomide (125–150 mg/m2, 7days on/7days off) and daily lapatinib (1250 mg/day). Efficacy was based on serial imaging of either brain or spine using MRI. Clinical benefit was evaluated using longitudinal assessment of symptom burden (MDASI-BT/MDASI-Spine) and performance status. Primary endpoint was progression free survival. Additional endpoints included 12-month PFS rate, objective response and clinical benefit (KPS and changes in proportion of moderate-severe disease-related symptom burden through cycle 6). RESULTS: 50 patients were treated with this regimen. Median age = 43.5, median KPS = 90, median number of prior relapse = 2. By tumor grade: III n = 19, II n = 16, I n = 8. 25 patients had spinal cord, 15 had supratentorial and 8 had infratentorial tumors. Treatment was well tolerated with a 10% Grade 3–4 myelotoxicity rate. Median PFS = 0.65 years (95%CI 0.46, 1.02). 1-year PFS=38%, with 1 complete and 4 partial responses. KPS improved in all but 1 patient and MDASI measures were completed on 86% of cases with reduction in moderate-severe pain in 53% and 71% of brain and spine patients respectively. Patients with spine tumors reported improvement in numbness (57%), weakness (24%), fatigue (23%)and autonomic dysfunction (bladder control (73%), sexual function (9%), and bowel function (36%)). CONCLUSIONS: These results demonstrate evidence of clinical activity, including objective responses and a nearly 40% stable disease rate at one year, with improvement in disease-related symptoms. This combination regimen was well tolerated and should be considered as a standard salvage regimen for adult patients with recurrent ependymoma.

RARE-26. MUTATIONS IN MAPK PATHWAY GENES ARE CHARACTERISTIC AND CONFIRMATORY OF MULTINODULAR AND VACUOLATING NEURONAL TUMOR OF THE CEREBRUM

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BACKGROUND: Multinodular and vacuolating neuronal tumor of the cerebrum (MVNT) is a rare, recently described neoplasm in the 2016 World Health Organization (WHO) classification with 14 cases reported to date. MVNTs usually present with epilepsy or are discovered incidentally. Median age of diagnosis is 44.9 years (22 to 71 years), and the most common location is the temporal lobe (~65%). METHODS/RESULTS: After reviewing 282 patients enrolled in our natural history study between September 2016 and August 2017, we uncovered two additional cases: the first patient is a 39-year-old woman and the other is a 57-year-old man. The tumors were localized in the left temporal and left frontal lobes respectively. Gross total resection was performed in both cases. Histological features were consistent with the 2016 WHO classification and other published reports. A CNS specific molecular panel revealed a BRAF p.Leu597Arg missense mutation in our first patient, a mutation that was recently reported in one other MVNT but has not been detected in any other primary CNS neoplasm within our database. The second patients lesion was found to have MAP2K1 p.Gln56Pro missense mutation. Our literature review found 2 cases with BRAF and 5 cases with MAP2K1 mutations in MVNT. MAPK pathway alterations reported in other glioneuronal tumors include BRAF p.Val600Glu mutations as well as BRAF fusions, neither of which have been reported in MVNTs to date. CONCLUSIONS: MVNT while rare, will likely be increasingly recognized. Our results suggest that the BRAF p.Leu597Arg missense mutation, (now described in two cases) and the MAP2K1 p. Gln56Pro missense mutation, may be useful diagnostic adjuncts to histopathological features and in differentiating this entity from more commonly epilepsy associated glioneuronal tumors.

RARE-27. CHIMERIC SPINAL CORD GLIOPROLIFERATIVE LESION FOLLOWING INTRATHECAL FETAL STEM CELL INFUSION

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BACKGROUND: Commercial stem cell therapy is available in select practices, particularly at non-U.S. sites, and is increasingly sought as a therapy by patients who are willing to travel for off-label treatment. To date, two cases of a spinal cord "glioneuronal lesion" or "glioproliferative lesion" have been reported. We report the third case, which by microdissection showed the glioproliferative spinal cord lesion to have mixed host/donor origin. CASE: A 73- year-old man presented with 3–4 years of progressive lower extremity weakness; he received intrathecal stem cell therapy (at least partly fetal in origin) in Russia, 7/2016. Symptoms began about 1 month after infusion. At presentation to our clinic in 11/17, neuroimaging showed nerve root enlargement with mild enhancement and clumping of nerve roots. Cerebrospinal fluid demonstrated significant elevation of protein (>1500mg/dL) and low glucose (32mg/dL). Multiple CSF samples were negative for malignancy or clonality. Extensive laboratory work-up for malignancy and inflammatory processes was negative. However, PET-CT showed intense uptake along the nerve roots of the cauda equina. Nerve root biopsy on 1/4/2018 showed a glioproliferative lesion with moderate cell density and

cytological atypia associated with mononuclear inflammation. Microdissection of the inflammatory element showed only host cells while alleles from two different individuals, one the recipient, were identified in the glioproliferative cord process. With progressive symptomatology, the patient received 2 cycles of high dose methotrexate. Follow-up PET-CT showed progression and he was referred for radiation therapy. CONCLUSION: This case with identification of chimeric origin for the glioproliferative, but not inflammatory, component of the lesion builds on the two previously-reported cases and underscores the need for greater awareness of potential complications of commercial stem cell therapy.

RARE-30. CASE SERIES OF INTRACRANIAL NON-GERMINOMATOUS GERM CELL TUMOR: A SINGLE INSTITUTION EXPERIENCE

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Intracranial non-germinomatous germ cell tumors are a rare, heterogenous group of neoplasms, commonly occurring in the pineal and/or suprasellar regions. The subtypes include yolk sac tumor (YST) or endodermal sinus tumor (EST), embryonal carcinoma (EC), choriocarcinoma (CHC), and mixed malignant subtypes. Diagnosis is usually made without a biopsy, through detection of elevated levels of Alpha-fetoprotein (AFP) and/or beta-human chorionic gonadotropin (βHCG) in the serum and/or cerebrospinal fluid. Three patients with intracranial NGGCT were treated at our institution, using a combination of surgery, chemotherapy, and/or radiation therapy. The clinical presentation of all cases included signs and symptoms of obstructive hydrocephalus. Patient #1 was diagnosed with malignant teratoma at birth via neuroimaging; she died from complications due to surgical resection. Patient #2 was diagnosed with choriocarcinoma of the pituitary gland at age 5 years via elevated AFP in serum and CSF; he was treated with alternating cycles of carboplatin/etoposide and ifosfamide/etoposide, followed by whole ventricle radiation therapy. He is now 28 months off-therapy, without evidence of disease. Patient #3 was diagnosed with choriocarcinoma of the pineal and suprasellar regions at the age 7 years, via elevated beta-HCG in serum and CSF, with evidence of bimodal disease. Treatment included 6 cycles of risk-adapted chemotherapy followed by whole ventricle radiation therapy. Neuroimaging continues to show no evidence of disease with normalization of tumor markers, at 24 months off-therapy. Patients #2 and #3 have residual neuroendocrine and neuropsychological deficits, but remain neurodevelopmentally appropriate. Combination of chemotherapy and radiation therapy is required for optimal prognosis.

RARE-31. NATURAL HISTORY OF T-CELL CNS LYMPHOMA

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BACKGROUND: Intravascular T-Cell Lymphoma is a rare form of non-Hodgkin's lymphoma. The clinical manifestations, treatment, and outcomes are poorly characterized. PURPOSE: This article provides a comprehensive analysis of intravascular T-Cell lymphoma from 1986–2018 to better understand this disease. METHODS/ANALYSIS: Studies from PubMed, Cochrane Review, and Springer were selected if patients demonstrated histologically or biopsy proven disease. Information on demographics, histology, site of disease, imaging, EBV exposure, and treatments was compiled. Univariate Cox proportional hazards model was used to analyze impact of these single variables and survival. Overall survival KM estimate was plotted. RESULTS: 56 unique patients (median age: 59, range: 0–87) were identified. Males were more affected (58.9%), with the most common primary disease manifestation being the skin (51.7%). Patients who were alive during time of study publication were in the minority (30.4%). Median time to diagnosis was 2.1 (range 0–18) months, with median time from first treatment to death at 3.1 (0–10) months. The hazard odds ratio for primary site skin involvement versus other sites was 2.198 (p=0.0271). The remaining independent variables, including treatment therapies, were not shown to be statistically significant. Survival KM estimate showed a survival function of 0.22 at 20 months. CONCLUSION: Intravascular T-Cell Lymphoma is a devastating disease, characterized by rapid onset and low survival rates. With the exception of initial site of presentation, no other variables or therapies have shown to improve prognosis and outcomes. It is imperative to continue to study this rare disease to mitigate its devastating effects.

RARE-32. EXTRA-ARTICULAR TENOSYNOVIAL GIANT CELL TUMOR OF DIFFUSE TYPE IN THE TEMPORAL AREA WITH BRAIN PARENCHYMAL INVASION

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Tenosynovial giant cell tumor of diffuse type is a locally aggressive neoplasm that most commonly arises in the lower extremities. However, pure extra-articular TGCT of diffuse type invading the brain parenchyma of the temporal area without involvement of the joint has not been reported to date. Herein, we report for the first time a case of an extra-articular tenosynovial giant cell tumor of diffuse type in the temporal region with brain parenchymal invasion. Imaging studies revealed an intracranial expansile mass in the temporal bone without involvement of the temporomandibular joint. The unusual location of the tumor without involvement of the joint and the presence of brain parenchymal invasion made this case challenging to diagnose. The patient underwent temporal craniotomy, and the mass was completely removed. The patient underwent temporal craniotomy, and the mass was completely removed. The tumor had invaded the cerebral parenchyma of the temporal lobe; it showed adhesion to the adjacent dura and no association with the adjacent joint. The cut section of the mass was solid and yellow to brown with necrosis and hemorrhage. Microscopically, the tumor showed densely cellular sheets of mononuclear cells, irregularly distributed osteoclast-like giant cells, and hemosiderin pigments. Specifically, the mononuclear cell population was composed of small polygonal or spindle cells with pale eosinophilic cytoplasm and large mononuclear cells with ovoid or kidney-shaped nuclei displaying prominent nucleoli and vesicular chromatin, which exhibited little pleomorphism. The tumor cells were positive for CD68 on immunohistochemical staining. Most extra-articular tumors present as a periarticular mass with frequent involvement of the adjacent joint and cystic lesions in the adjacent bone, although on rare occasions, these lesions can be purely extra-articular. Wide excision is the treatment of choice; however, complete excision of the lesion is often impaired by the need to save an adjacent joint. Therefore, radiotherapy is recommended.

RARE-35. LONG-TERM RESULTS OF HIGH-DOSE METHOTREXATE TREATMENT FOR PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA. A MULTI-INSTITUTIONAL EXPERIENCE

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INTRODUCTION: In spite of multi-modality treatments, primary central nervous system lymphoma (PCNSL) has been a challenging tumor. For a long time, high-dose methotrexate (HD-MTX) is used as a first option for the treatment. Here, we present the experience of HD-MTX for PCNSL with one protocol in 3 institutions and suggest the prognostic factors. METHOD: From 2001 to 2016, 68 patients with PCNSL was treated with HD-MTX in 3 hospitals in Republic of Korea. The protocol of HD-MTX was composed with the 3 cycles of induction chemotherapy and 6 cycles of maintenance chemotherapy. Data was collected retrospectively and Kaplan-Meier method was used to analyze the survival. RESULTS: The scheduled 3 cycles of induction chemotherapy with HD-MTX was performed in 53 patients and maintenance chemotherapy was performed in only 26 patients. Good radiologic response was shown in 39 patients (57.4%) after induction chemotherapy, and overall radiologic responses after the completion of HD-MTX chemotherapy were complete remission in 24 (35.3%), partial response in 3 (4.4%), stable disease in 2 (2.9%), progressive disease in 24 (35.3%), and unknown in 15 patients (22.1%). The recurrence was evented in 16 patients among the good response patients, and median recurrence free period was 24.6 months (95%CI; 22.4–26.8 months). The median overall survival of our patients treated was 51.8 months (95%CI: 39.7–63.9). The patients with age less than 70 years old, the good radiologic response after induction HD-MTX chemotherapy, or the over 3 cycles of induction chemotherapy showed the long overall survival time significantly (p CONCLUSION: Although this study was performed retrospectively, the response of HD-MTX was significantly related to age and the response and number of cycles of induction chemotherapy. We suggest that the HD-MTX is still the ponderable treatment as the first therapy for PCNSL.

RARE-36. MALIGNANT PHEOCHROMOCYTOMA METASTATIC TO THORACIC VERTEBRA: PALLIATIVE NEUROSURGICAL MANAGEMENT

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OBJECTIVE: Describe a rare case of metastatic pheochromocytoma to thoracic vertebra and the neurosurgical management with palliative objec-

tive in the context of malignant pheochromocytoma. BACKGROUND: Up to 10% of pheochromocytomas can be malignant. Bone metastases are an usual site of dissemination with some reports of rates up to 71%. However, the involvement of vertebra its very rare, Liu et al reported that in November 2017 only exist 17 reported cases of vertebral metastases of malignant pheochromocytoma. Also, within the thorax the most common places for paragangliomas are the mediastinum and, in second place, the heart. For this kind of pheochromocytoma, the survival rate to five years is close to 40% to malignant, and there is no curative treatment, and do not exist reliable data about the improvement of survival rates given by surgical management of metastatic lesions, that is the reason why the surgical treatment is focus in prevent local complications given by the metastatic disease. This case is about a 40 year old male with previous diagnosis of pheochromocytima treated surgically in the past, who presented now with precordial pain. In diagnosis studies was ruled out cardiac disease, and confirmed progression of disease to sternum, and incidentally findings in T4 vertebra. The patient doesn't present neurological symptoms. The case were presented in the weekly meeting for neurooncology decision making, in which was decided to perform a palliative surgery consisten in medullar decompression and arthrodesis two levels above and below the lesion. RESULTS: Surgical pathology revealed paraganglioma with chromaffin cells. CONCLUSION: Malignant pheochromocytoma is a rare entity, with common metastases to bone. Once the metastases is stablished, there is no curative treatment for the disease and the treatment should be focus to ameloriate the quality of life and prevent local complications, in this case, medullary compression.

RARE-37. OCCURRENCE OF GLIOMA IN PREGNANT PATIENTS: INSTITUTIONAL CASE SERIES AND REVIEW OF THE LITERATURE

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BACKGROUND: The treatment of glioma in pregnant patients has limited data in the literature. There are no guidelines in place currently for this population. Another controversial topic is the use of gadolinium contrast in pregnant patients. This retrospective institutional case review series looks at the extent of resection, grade of tumor, use of contrast and outcome. This case series is the first to document IDH-mutation and *MGMT* methylation status either by immuno-histological stain or by genetic sequencing. METHODS: 5 patient cases were reviewed by retrospective search with diagnosis of glioma and pregnancy. 4 patients were diagnosed with glioma during pregnancy; 1 patients were diagnosed with glioma prior to pregnancy. 3 patients were monitored with contrasted MRIs after 2nd trimester, there were no adverse effects to the fetus. Only 1 patient developed progression during course of chemotherapy, the remaining 4 patients remained progression free after completion of chemotherapy. RESULTS: This case series documented that 3 of 5 patients had IDH-mutated gliomas; 1 of 3 glioblastomas (GBM) were *MGMT* methylated. 3 of 5 patients achieved gross total resection. All intracranial neoplasms were of astrocytic lineage: 3 GBMs, 1 anaplastic astrocytoma and 1 diffuse astrocytoma. All patients delivered healthy infants. 4 patients continue to be progression free since completion of chemotherapy with 1 patient experiencing progression during course of chemotherapy. Median progression free survival was 20 months, all patients are alive at time of publication. CONCLUSION: This case series highlights how individualized care through a multi-disciplinary approach can result in favorable outcomes in this patient population.

RARE-40. PERSONALIZED MEDICINE DIAGNOSIS AND RESPONSE TO TREATMENT WITH BRAF MEK INHIBITION IN A PLEOMORPHIC XANTHOASTROCYTOMA PATIENT

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OBJECTIVE: To highlight personalized medicine in a patient with a brafmt Pleomorphic Xanthoastrocytoma (PXA) treated with BrafMEKi. BACKGROUND: PXA is a rare low-grade astrocytoma. Rarely (as in this patient) may be malignant, accounting for less than 1% of all CNS neoplasms. BRAF-V600E mutation is usually found in PXA. Clinical trials showed BRAF tyrosine-kinase inhibitors as viable options to treat metastatic brain melanoma tumors. Recent studies showed similar therapy efficacy in primary brain tumors with BRAF mutations. Other studies suggest that brain tumors with BRAF-V600E mutation that fail BRAF inhibitors can benefit from chloroquine, an autophagy inhibitor, by overcoming the resistance of the kinase inhibitors. CASE REPORT: A 20 year-old man presented with a headache. MRI showed a large lesion, which appeared unusual for a classical Glioblastoma Multiforme (GBM). He was diagnosed with "GBM" and treated with radiation/temozolomide. Follow-up imaging showed recurrence. Histology at Moffitt showed an atypical malignant glioma that resembled PXA. Molecular testing demonstrated a BRAF-V600E mutation. Based on this, targeted therapy to inhibit Braf/Mek with Dabrafenib/Trametinib was initiated. Subsequent MRI showed decrease lesion size. After eight-months, he was given a chemotherapy holiday; however, repeat imaging showed disease progression and treatment was re-started. His disease progressed months later, and chloroquine was added to chemotherapy. Follow-up imaging showed tumor remained stable, suggesting addition of chloroquine to Dabrafenib/Trametinib halted tumor progression. Patient remains under surveillance. CONCLUSION: A molecular diagnosis of PXA was made guiding targeted therapy. Molecular testing provides new avenues of diagnosis and treatment for NeuroOncology patients. Molecular interrogation should be routine for all NeuroOncology patients who are young or with unusual tumors. Addition of chloroquine overcame resistance to BRAF inhibition in this patient with a brain tumor with BRAF-V600E mutation.

STEM CELLS

STEM-01. PROSPECTIVE ANALYSIS OF CANCER STEM CELL DRUG RESPONSE ASSAY FOR GLIOBLASTOMA PATIENTS

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BACKGROUND: Over the past 20 years even with the aggressive standard of care (SoC) Stupp treatment protocol the prognosis of glioblastoma (GBM) has only minimally improved from 12 to 14 months. This is due in large part to the presence of chemo- and radiation-resistant GBM cancer stem cells (CSCs) that contribute to tumor propagation, maintenance, and treatment resistance. We are using ChemoID, a CLIA certified and CAP accredited drug response assay to identify the most effective chemotherapy treatment against CSCs and bulk of tumor cells from of a panel of chemotherapies, offering great promise for individualized cancer management. A prospective study was conducted evaluating the use of the ChemoID drug response assay in glioblastoma patients. METHODS: Fresh tissue samples were collected for drug sensitivity testing from 61 glioblastoma patients enrolled in IRB approved protocol. Patients were prospectively monitored for tumor response, time to recurrence, progression-free survival (PFS), and overall survival (OS). Odds Ratio (OR) associations of 12-month recurrence, PFS, and OS outcomes were estimated for CSCs, bulk tumor and combined assay responses to treatment; sensitivities/specificities, areas under the curve (AUC) were examined. RESULTS: The data suggests that ChemoID guided treatment significantly enhanced tumor response. For every 5% increase in cell kill of CSCs by assay-guided chemotherapy, 12-month patient response (non-recurrence of cancer) increased 2.5-fold, OR=2.3 (p=0.01). Bulk of tumor assay was found not statistically significant. Median recurrence time was 20 months for patients with a positive (>40% cell kill) CSCs test versus only 3 months with a negative CSCs test, whereas median recurrence time was 13 months versus 4 months for patients with a positive (>55% cell kill) bulk test versus negative. Similar favorable results for the CSC test were observed for PFS and OS outcomes. CONCLUSIONS: The ChemoID CSCs drug response assay has the potential to increase the accuracy of bulk tumor assays to help guide individualized chemotherapy choices. Glioblastoma cancer recurrence may occur quickly if the CSC test has a low cell kill rate, even if the bulk tumor test cell kill rate is high.

STEM-02. DEVELOPING BRAIN METASTATIC TUMOR MODELS FOR TARGETED STEM CELL THERAPY

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Metastatic brain tumors are the most commonly observed intracranial tumors, and 10–20% of adults with cancer develop brain metastasis. Characterizing clinically relevant brain metastasis models and exploring different delivery options to deliver drugs across blood brain barrier in such models are fundamental for the development of novel therapies for metastatic brain

cancers. We have created imageable breast to brain metastasis tumor models using patient derived brain seeking triple negative breast cancer (TNBC) lines. We show a widespread distribution of micro- and macro-metastasis and the close association of tumor cells with blood vessels in the brain thus mimicking the multi-foci metastases observed in the clinics. Next, we assessed the EGFR and DR4/5 levels in TNBC and show that brain seeking TNBC have higher EGFR and DR5 levels than their primary counterparts. We also show that intracarotid artery injection of stem cells engineered to express optical imaging markers home to metastatic brain tumors. These findings provide useful models for testing treatments for metastatic brain tumors and also provide insights into the changes in gene expression in metastatic TNBC and means to deliver therapeutics to metastatic tumors in the brain.

STEM-03. NOVEL METASTATIC BRAIN TUMOR TARGETS ISOLATED THROUGH PHAGE DISPLAY BIOPANNING AGAINST BRAIN METASTASIS-INITIATING CELLS

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Despite treatment advances for primary cancers that may result in brain metastases, the treatment for brain metastases has lagged behind, partly due to the unique environment created by the central nervous system. Recent work has highlighted that metastatic brain tumors demonstrate significant genotypic deviation from their parental tumors, which highlights the need to focus on metastatic brain tumors for the development of more effective targeted therapeutics. The epithelial-mesenchymal transition suggests the existence of brain metastasis-initiating cells (BMICs), which may possess stem cell-like phenotypes that play a pivotal role in metastasis. To properly isolate this subpopulation of tumor cells, we compared patient derived metastatic brain tumor cells against brain metastasis cell lines. We found that both types of cells demonstrated similar morphology when grown in standard serum media conditions, but when grown in serum-free media, both demonstrated a tumor sphere morphology. Gene expression analysis showed increased expression of stem cell markers CD133 and EYA1 when grown in serum-free media. To isolate therapeutic targets specific to BMICs, we performed *in vitro* and *in vivo* phage display biopanning against BMICs. Analysis of the recovered peptides derived several potential targets. Among them were angiomotin (AMOT) and bromodomain testis-specific factor (BRDT). Gene expression analysis confirmed significant upregulation of these gene in metastatic brain tumors cells when compared to non-tumor neural cells and primary lung cancer cell lines. Tissue microarray analysis of eleven matched brain metastasis and primary lung cancer patient tissue from Moffitt Cancer Center demonstrated an increased expression in brain metastasis over primary lung tissue. Analysis through NIH GEO database demonstrated decreased survival with increased gene expression. Our results show that we can use phage display biopanning to isolate novel targets that may be specific to metastatic brain tumors by isolating the proper growth conditions that support the BMIC phenotype.

STEM-04. GLIOMA STEM CELL REGULATOR S100A4 MODULATES GBM IMMUNE LANDSCAPE

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Glioblastoma (GBM) is the most common and aggressive malignant brain tumor in adults and is virtually incurable. Immunotherapy is a promising new approach to treat GBM as it harnesses one's own immune system to recognize and kill aberrant cancer cells. Unfortunately ongoing trials with immunotherapies show disappointing results in most glioma patients. GBM has highly immune-suppressive microenvironment. Consistently, mesenchymal subtype, the subtype with worst prognosis, has a strong immune signature. We recently reported that S100A4 is necessary for human and mouse glioma initiating cell (GIC) self-renewal and tumor growth, and that S100A4 is a master regulator of mesenchymal transition in GBM. Importantly, we report that S100A4 regulates expression of cytokines that affect TAM infiltration and polarization towards tumor-promoting phenotype. Consistently, TCGA and IVY-GAP data analyses indicate that S100A4 expression is strongly correlated with GBM patient survival, the mesenchymal subtype, and tumor-promoting TAM (tumor-associated macrophage) and MDSC (myeloid-derived suppressor cell) marker expression (such as CD163, CD204/MSR1, IL10, CD11b, S100A8, and S100A9). S100A4 expression and TAM marker expression strikingly overlap in perivascular and perinecrotic regions, previously reported niches for GICs. Interestingly there is no correlation between S100A4 expression and markers of microglia. Through single cell RNA-sequencing analyses of human GBM samples, we now have evidence that S100A4 is expressed in both glioma cells and infiltrating myeloid cells. When S100a4 is knocked down in mouse glioma cells and transplanted into syngeneic mice, tumor promoting myeloid cell numbers are significantly reduced. We are currently testing the role of S100A4 in bone marrow derived myeloid cells and elucidating the molecular mechanism of S100A4 function. Our unpublished observations strongly suggest that S100A4 is a critical regulator of GBM immune landscape and may be key node that links GICs, mesenchymal transition, and the myeloid cell infiltration.

STEM-05. IDENTIFICATION OF PATIENT-DERIVED GLIOBLASTOMA STEM CELL (GSC) LINES WITH THE ALTERNATIVE LENGTHENING OF TELOMERES PHENOTYPE

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BACKGROUND: Telomeres are terminal DNA elements found at chromosomal ends consisting of hexagonal repeats of $(TTAGGG)_n$, which are essential for maintaining genomic stability. In glioblastoma, *TERT* promoter mutations occur in approximately 60–80% of cases, leading to increased telomerase activity and enabling replicative immortality. The remaining tumors, instead, activate a telomerase-independent alternative lengthening of telomeres (ALT) mechanism, driven by homologous recombination machinery. ALT+ gliomas are enriched in tumors harboring mutations in *ATRX* and *IDH*, and mutually exclusive with *TERT* promoter mutations. METHODS: A novel quantitative PCR (qPCR) method that measures both telomere content (TC) and extra-chromosomal telomeric DNA C-Circles (CCs) was used to screen a panel of 24 human GSC cell lines for potential ALT lines. mRNA expression of *TERT* (which encodes the catalytic component of telomerase) was measured via whole transcriptome sequencing. ATRX protein expression was assessed by immunoblotting. RESULTS: We identified 2 ALT+ GSC cell lines: GSC-522 and GSC-818. These 2 cell lines have significantly elevated DNA CC content ($p<0.001$) and telomere content ($p<0.001$) relative to the other GSCs, consistent with the ALT phenotype. Furthermore, whole transcriptome sequencing identified mRNA expression of *TERT* to be negligible in these two cell lines relative to the others ($p=0.0023$), suggesting absence of telomerase activity. Both GSC-522 and GSC 8–18 were found to lack detectable full length ATRX protein upon immunoblot analysis, suggesting loss of function of this pathway. CONCLUSIONS: Identification of ALT+ GSCs will enable future explorations of the mechanisms and biology of the ALT phenotype in the context of glioma, and will also help define radiation sensitivity and resistance patterns in ALT vs. telomerase-positive gliomas. Furthermore, these cell lines will serve as pre-clinical models to test novel chemotherapeutic agents in an effort to improve outcomes in a subset of high-grade gliomas.

STEM-06. A DRAFT SINGLE-CELL ATLAS OF HUMAN GLIOBLASTOMA REVEALS SPATIAL AND DIFFERENTIATION GRADIENTS OF STEM-LIKE CELLS

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Glioblastoma (GBM) stem-like cells (GSCs) expressing markers of the proneural (e.g. OLIG2, DLL3) and mesenchymal (e.g. CHI3L1(YKL-40), CD44) GBM molecular-subtypes have been described. However, it is unknown if these two GSC types are sufficient to generate the spectrum of cellular heterogeneity observed in GBM. The spatial localization and niche interactions of GSCs have not been fully elucidated. We perform single-cell RNA-sequencing (scRNA-seq) and matched exome sequencing of human GBMs from a cohort of 21 patients, profiling over 40,000 cells. We combine in silico lineage tracing and meta-analysis of public data to identify recurrent cellular hierarchies of GSCs and their progeny. We map GBM cells to tumor-anatomical structures using reference atlases. We perform immunofluorescence staining and automated image analysis on a cohort of 300 human GBMs using markers of GSC lineage and phenotype to visualize and quantify niche interactions. Proneural GSCs (pGSCs) upregulate markers of cell-cycle progression and PDGF signaling compared to mesenchymal GSCs (mGSCs). By contrast, most mGSCs have a quiescent phenotype, but overexpress cytokines responsible for the chemotaxis of myeloid-derived suppressor cells (e.g. CSF1, CCL2, PTGS2). We find cycling mGSCs enriched in the perivascular space and quiescent mGSCs enriched in hypoxic regions, pGSCs are enriched in the tumor's invasive edge. Increased mGSC con-

tent, but not increased pGSC content correlates with significantly inferior survival in TCGA data. All clonal expressed-mutations in our biopsies are found in the GSC populations, with a greater representation of mutations in mGSCs. We conclude that varying proportions of mGSCs and pGSCs, their progeny and stromal/immune cells are sufficient to explain the genetic and phenotypic heterogeneity observed in GBM. Moreover, pGSCs and mGSCs aggregate in distinct tumor compartments, display different rates of proliferation and distinct niche interactions. Intratumor heterogeneity is a barrier to targeted therapeutics. This study sheds light on the heterogeneity of GSCs in vivo.

STEM-07. NON-CANONICAL REGULATION OF SOX2 BY THE TRIM26 E3 UBIQUITIN LIGASE IN GLIOBLASTOMA STEM-LIKE CELLS

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Ubiquitin-dependent signaling is critical for the regulation of glioblastoma stem-like cells (GSCs), a subpopulation of glioblastoma cells responsible for tumor growth and therapy resistance. TRIM26, an E3-ubiquitin ligase with immune-related functions, is highly expressed in glioblastoma tumors compared to normal brain. SOX2 immunoprecipitation followed by mass spectrometry in a patient-derived primary GSC line suggested that TRIM26 interacts with SOX2, a key pluripotency-related transcription factor essential for GSC maintenance. We hypothesized that TRIM26 may play a critical role in GSC maintenance by regulating SOX2. We found that TRIM26 and SOX2 interact directly via the C-terminal PRY-SPRY domain of TRIM26 and the DNA-binding HMG-box of SOX2. Unexpectedly, TRIM26 overexpression resulted in decreased SOX2 polyubiquitination in HEK293 cells. In line with this observation, TRIM26 knockdown in GSCs decreased ambient SOX2 protein levels and stability. Accordingly, SOX2 transcriptional activity, as assessed by a transcriptional reporter, was diminished in the setting of TRIM26 RNA interference (RNAi). Functionally, TRIM26 knockdown reduced GSC self-renewal *in vitro* and tumorigenicity *in vivo* in a xenotransplantation mouse model. Mechanistically, TRIM26 competitively reduced SOX2 interaction with WWP2, a bonafide SOX2 E3 ligase in GSCs. Moreover, WWP2 knockdown in GSCs restored SOX2 protein expression in the setting of TRIM26 RNAi. Upstream of TRIM26, we found that tumor-associated macrophage-derived TGFb1 increases TRIM26 expression in GSCs. Together, these results suggest that TRIM26 maintains GSCs by protecting SOX2 from WWP2-mediated ubiquitination and subsequent proteasomal degradation, and further that this pathway may be subject to regulation by the tumor microenvironment.

STEM-08. MODULATION OF RADIATION-INDUCED MESENCHYMAL STEM CELL MIGRATION IN GLIOBLASTOMA

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INTRODUCTION: Glioblastoma is the most common primary adult brain tumor and carries a devastating prognosis. Mesenchymal stem cell (MSC) therapies offer a new strategy that could provide a novel treatment approach; however, it is unknown how MSC therapy should be integrated into the current standard of care: radiation and chemotherapy. AIMS: Study the modulatory effect of ionizing radiation (IR) on MSC migratory patterns. METHODS AND RESULTS: To confirm IR-induced lethality, alamarBlue assay of radiated patient-derived glioblastoma brain tumor initiated cells (BTICS) showed decreased viability after 10 Gy and 20 Gy IR when measured at 48 hours (p=0.006). MSCs were transduced with a lentiviral vector for expression of iRFP and, *ex vivo* implantation of MSCs was carried out on organotypic slices of normal mouse brain to evaluate engraftment and migration in the setting of IR delivery, groups of 0 Gy, 2 Gy, 5 Gy and 10 Gy were compared via confocal microscopy using 3D time lapse and daily qualitative evaluation of migratory patterns. Migratory distances showed a positive correlation with increasing dose of IR compared to control. Additionally, to assess the effect of IR on MSC migration, transwell assays were performed, in quadruplicate, using the radiated conditioned media from patient-derived glioblastoma BTICs; after incubating for 24 hours, migration was assessed by directly counting DAPI-labeled nuclei via fluorescent microscopy. Reduced MSC transwell migration was found after 10 Gy IR compared to the control, 2 Gy IR, and 5 Gy IR groups (p=0.008, p=0.002, and p=0.010, respectively). CONCLUSION: MSC migration to BTIC-conditioned media decreases when BTICs are exposed to high dose IR, however migration of MSCs seems to follow a positive relationship with the delivery of IR on an *ex vivo* mouse model. This suggests that low dose, but not high dose, radiation could 'prime' the tumor microenvironment for MSC delivery.

STEM-09. CANCER STEM CELL IMMUNOEDITING

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INTRODUCTION: Conventional therapies most effectively eliminate highly proliferative tumor cells but leave behind residual slow-cycling cancer stem cells (SCCs) that evade treatment and ultimately seed disease recurrence. We reported the existence of SCCs that exhibit enhanced invasiveness, tumorigenicity and resistance to therapy in glioblastoma (GBM). The increased tumorigenicity of these GBM SCC suggests they may have enhanced immunoediting capacity. OBJECTIVES: We speculate that GBM SCCs drive disease progression and recurrence through immune escape. The objective of this study is to characterize the GBM SCC interface with the immune system and identify immunosuppressive mechanisms employed by these cells. METHODS: SCCs were isolated from mouse and human models of malignant glioma and separated by flow cytometry based on their ability to retain CellTrace labeling. RESULTS: Draining lymph nodes from animals bearing SCC-derived malignant gliomas showed decreased $CD8^+$ and increased $CD4^+/Foxp3^+$ cells. Immune gene signature analysis of tumor infiltrates uncovered enhanced recruitment of macrophages to SCC-derived tumors, with ApoE being the most over-expressed. ApoE promotes macrophage polarization toward the immunosuppressive M2 phenotype and inhibits T cell interferon-(IFN)-γ secretion. Using the IFN-γ reporter (GREAT) mice, we found that tumors derived from SCCs repressed IFN-γ secretion/expression by *in vivo* imaging, ELISA and RNA sequencing. This correlated with the up-regulation of gene networks in SCCs involved in immunosuppression, specifically CSF1 a prominent survival factor for macrophages. CONCLUSION: The immune surveillance hypothesis suggests that oncogenesis, driven by cancer cell immunoediting, shapes the immune system toward a pro-tumorigenic phenotype. Our study indicates that SCCs play a pivotal role in shifting the GBM immune milieu toward a regulatory phenotype characterized by M2 macrophages polarized by ApoE signaling.

STEM-10. MOLECULAR MECHANISMS UNDERLYING PHENOTYPIC PLASTICITY IN MALIGNANT GLIOMA

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Glioblastoma (GBM) is characterized by rapidly proliferating and invasive cells that infiltrate normal brain regions. Following exposure to aggressive treatment regimens, GBMs frequently shift their biological features upon recurrence, acquiring a more resistant phenotype. However, the dynamics and molecular mechanisms that facilitate GBM recurrence are still poorly understood. The objective our study was to determine how glioma stem cells (GSCs) temporally adjust their expression profile and phenotype in response to ionizing radiation or Temozolomide (TMZ) *in vivo* using patient-derived xenograft (PDX) models of GBM. We established two PDX GBM models by intracranially implanting two patient-derived GSC lines belonging to different GBM molecular subgroups (proneural and classical) into immunocompromised mice. The tumor-bearing mice were treated with single or multiple doses of ionizing radiation or TMZ to assess acute and long-term responses to treatment respectively. Mice from each cohort were be sacrificed at multiple distinct time points following treatment. Using immunohistochemical methods, we assessed changes in the expression of GBM subclass markers, stemness and differentiation markers, and DNA damage/repair proteins across the entire tumor population over time. Furthermore, to understand how the PDX tumors respond to radiation at a molecular level, we employed mass cytometry (CyTOF) and ChIP-seq to determine how important cellular signaling pathways and transcriptional programs necessary for GSC self-renewal, invasion and growth are altered at various time points post-treatment. We demonstrate that GSCs undergo an immediate response following exposure to radiation that results in a global modulation of the expression of key stemness and proliferation genes under adverse conditions. Our results

suggest that this acute response allows GSCs to enter a transient semi-differentiated state that favors GSC adaptability and resistance to therapy.

STEM-11. DIRECTED NEURONAL DIFFERENTIATION AS A THERAPEUTIC STRATEGY FOR MALIGNANT GLIOMAS

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IDH-mutant (IDH-MUT) glioma patients show highly improved prognosis compared to those with wild-type IDH. However, IDH mutations are rare in the more aggressive grade IV glioblastomas and introducing IDH mutations in mouse neural cells does not induce gliomagenesis. This is a paradox, given that IDH mutation is considered an early oncogenic event and the mutated IDH produces the oncometabolite 2-hydroxyglutarate. Here we identify a previously unknown mechanism that explains this paradox. We found that IDH-MUT patient tumors expressing a molecular signature enriched in genes associated with neuronal differentiation survive the longest (~11 years) and these same tumors have significantly lower expression of the Mesenchymal master regulator TAZ, which is epigenetically silenced. Concordantly, IDH-MUT/TAZ-low glioma stem-like cells (GSCs) show remarkable neuronal differentiation induced by retinoic acid, whereas IDH-WT/TAZ-high GSCs are resistant to this process implying that IDH status and TAZ levels dictate propensity to neurogenesis. TAZ overexpression in IDH-MUT GSCs caused inhibition of retinoic acid neuronal differentiation and conversely CRISPR/Cas9-mediated knockout of TAZ in IDH-WT GSCs promotes this process as judged by alteration in genes regulating neurogenesis as well as cellular morphology resembling neurons (dendritic branching etc.). Preliminary data on the mechanism of TAZ inhibition, indicate that TAZ might directly act as inhibitor of gene expression via formation of a complex with HDAC-1 and -2. Verteporfin, a FDA approved inhibitor of TAZ caused reduction of tumor volume and improved survival in xenograft bearing mice. We are currently evaluating brain penetrating HDAC inhibitors for their ability to induce neuronal differentiation and tumor inhibition in intracranial models of GBM. Taken together, our studies uncover a role for TAZ as a barrier for terminal neuronal differentiation and that differentiation therapy TAZ or HDAC inhibitors is possible for IDH-WT gliomas if combined with retinoids.

STEM-12. DOWNREGULATION OF H-FERRITIN EXPRESSION USING MULTIVALENT CATIONIC LIPOSOMES RESULTS IN INCREASED RADIATION SENSITIVITY IN PATIENT DERIVED GLIOMA INITIATING CELLS

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Glioblastoma is the most prevalent and lethal primary brain tumor with a dismal survival rate. The glioma initiating cell (GIC) population within glioblastomas has been associated with treatment resistance and subsequently been implicated in tumor recurrence. Studies from our lab had previously shown that expression of the heavy chain subunit of the iron storage protein ferritin, H-ferritin, is essential for the survival and therapeutic resistance of non-stem glioma cells. Since H-ferritin is overexpressed in GICs, we hypothesized that downregulating its expression in these cells would lead to increased radiosensitivity. We thus developed a novel strategy to sensitize GICs to radiation therapy using a multivalent cationic liposome formulation that could efficiently transfect and deliver H-ferritin siRNA to GICs *in vitro*. Using patient derived pro-neural T3691 and mesenchymal T387 CD133+ GICs we showed that downregulating H-ferritin led to increased LDH release as well as executioner caspase 3/7 activity in both GIC subtypes. We also found a significant decrease in the levels of Tom 20, a mitochondrial outer membrane protein, indicating reduced mitochondrial mass. However, upon radiation at 8Gy we found that T3691 but not T387 cells showed a significant decrease in cell viability. Upon further investigation we found that knockdown of H-ferritin led to elevated expression of the DNA damage response protein phospho-ϒH2AX to a much higher extent in T387 GICs (9.2 fold) compared to T3691 GICs (3.3 fold) suggesting that these cells might be able to repair DNA damage more efficiently than the pro-neural subtype and thus remain radiation resistant even after H-ferritin loss. Thus, we have demonstrated that loss of H-ferritin in pro-neural GICs leads to increased radiation sensitivity presumably through induction of mitophagy and increased DNA damage. Ongoing studies are focused on further determining the mechanisms through which H-ferritin loss mediates this effect in pro-neural but not mesenchymal GICs.

STEM-13. HYPOXIC INDUCTION OF VASORIN MEDIATES GLIOMA STEM CELL-ENDOTHELIAL CELL INTERACTIONS IN THE PERIVASCULAR NICHE

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Glioma stem-like cells (GSCs) have been recognized to play an important role in tumor progression. GSCs are highly resistant to radiation and chemotherapy and have a high capacity to self-renew and differentiate into multiple lineages. GSCs preferentially reside within niches including the hypoxic and perivascular niches that help to support their undifferentiated state. Interactions between GSCs and different cell types in these niches, including endothelial cells, help to maintain GSCs and facilitate tumor migration, invasion and angiogenesis. We previously identified that the transmembrane protein Vasorin (VASN) is a critical node in GSC maintenance. Little is known about this protein (< 20 papers), but we have found that VASN is overexpressed in about 80% of GBM and is associated with poor prognosis. VASN is preferentially induced in GSCs in hypoxia and binds Notch1 to stabilize it at the cell membrane. This interaction effectively functions as a switch to amplify Notch signaling in GSCs in hypoxia to promote their survival. We now build upon these studies and find that VASN is expressed in GSCs not just in the hypoxic niche but also the perivascular niche. GSCs are abundant in the perivascular niche and endothelial cells can provide Notch ligands that help to maintain GSCs self-renewal properties. We have found that VASN is frequently expressed in hypoxic, CD44+ and Olig2+ tumor cells in the perivascular niche in GBM, and that VASN is expressed in a subset of tumor-associated endothelial cells. Our data suggest that reciprocal Notch signaling mediated by VASN between endothelial cells and GSCs in the perivascular niche helps to maintain GSCs. These data suggest that VASN may also play an important role in perivascular niche interactions between GSCs and endothelial cells through Notch signaling.

STEM-14. GROWTH FACTOR RECEPTOR CO-INHERITANCE DURING ASYMMETRIC CELL DIVISION DRIVES THE CANCER STEM CELL PHENOTYPE

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An asymmetric cell division (ACD) produces a stem cell and a differentiating progeny. Thus ACD ensures the generation of organs with heterogeneous cell populations without depleting pools of stem cells with regenerative capacity. Cancer stem cells (CSCs), which are similar to normal stem cells, can self-renew and regenerate tumors with cellular heterogeneity. CSCs are resistant to therapy and play a critical role in tumor recurrence. ACD has been detected in CSCs from many types of tumors, but its role in CSC fate decision has yet to be fully elucidated. A remaining technical limitation is that ACD is often defined retrospectively based on the observation of an asymmetric fate choice by CSC progeny. We previously demonstrated asymmetric inheritance of a surrogate CSC marker, CD133, during mitosis. To prospectively analyze the biological role of this inheritance asymmetry, we have developed a GFP reporter system capable of monitoring the degree of asymmetry of the CSC marker during mitosis. This reporter also revealed the asymmetric co-inheritance of growth factor receptors, the activation of which overrode the effect of a differentiation-inducing condition that suppresses self-renewal capacity and therapeutic resistance of CSCs. Preliminary time lapse-based lineage tracing detected that daughter cells that inherited higher levels of growth factor receptors based on GFP-reporter signal intensity express higher levels of a core stem cell transcription factor compared to their sister cells that inherited lower levels of growth factor receptors. These data suggest that asymmetrically co-inherited growth factor receptors promote the stem cell state in one of the progeny of a CSC undergoing ACD, ensuring the persistence of a therapeutically resistant population.

STEM-15. CDK5 AND NOTCH SIGNALING CROSSTALK DRIVES SELF-RENEWAL IN GSCs

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While the connection between stem cell biology, deregulation of self-renewal properties in glioma stem cells and involved signaling pathways which inherent and/or adaptive to tumor cell resistance has long been considered an important area of cancer research, studies on glioma stem cell self-renewal mechanisms has not always been forthcoming. In this regard, the role of atypical Cyclin Dependent Kinase 5 (CDK5) and its pathway in maintaining self-renewal properties has been characterized. CDK5 is a cyclin dependent kinase family member that does not regulate cell cycle directly. CDK5 is highly important for the survival of post-mitotic neurons and its aberrant activation has been instrumental in neurodegenerative diseases. Our research, as well as that of others, has identified CDK5 as a regulator of self-renewal in glioma stem cells (GSCs). In this capacity, the CDK5 directly activates CREB1 transcription factor independent of PKA/cAMP pathway to drive self-renewal. In the current study we have shown that CDK5 signaling is also a regulator of NOTCH1 activation. Notch pathway regulates self-renewal and asymmetric division through activation of Hes1 transcription factor. Notch pathway is also regulated by Numb and TRIM3 in GSCs and interestingly, TRIM3 regulates CDK5 activity. Our study unravels a signaling crosstalk between CDK5 and NOTCH1 in regulating self-renewal of GSCs.

STEM-16. STOX2, A NEW REGULATOR FOR GBM STEM CELL MAINTENANCE AND IMMUNE RESPONSE

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Glioblastoma (GBM) stem-like cells (GSCs) are highly radio- and chemoresistant and thought to be the main source of tumor relapses. Therapeutic targeting of GSC has vital significance. However, the mechanism regulating GSCs' survival remains incompletely understood, making eradicating GBM difficult to attain. Here, we describe a identification and characterization of a novel master regulator of GSCs discovered using a computational approach. STOX2 a little known protein was found to be upregulated specifically in GSC compared to normal neuronal stem cells and be exquisitely fundamental to the maintenance and survival of GSCs in general. We demonstrated in several patients derived GSC xenografts. that STOX2 depletion in GSCs quickly pushed cell into apoptosis, most likely through the downregulation of a key stem-ness program. Additionally, expression of PD-L1, an immune suppressing ligand, in GBM required high level of STOX2, indicating a dual function for STOX2 in maintaining GSC stem-ness while preventing them from being recognized by the immune system. Thus STOX2 is a novel GSC master regulator that is indispensable for GSC maintenance and may represent a new therapeutic target for the treatment of GBM.

STEM-17. SPATIAL IDENTITY OF GLIOBLASTOMA CELLS DEFINES THERAPY-INDUCED CLONAL COMPETITION

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Glioblastoma (GBM) is characterized by high tumor heterogeneity. It has been hypothesized that the tumor core is enriched with tumor cells harboring mesenchymal phenotype, whereas the tumor edge retains those with proneural phenotype. Recent studies including ours have identified the mutually-distinct glioma stem cells (GSCs) are present in these two lesions. Here, in order to investigate the diversity of GSCs, we established subclones of GSCs from the edge and core region of the tumor of GBM patients. These subclones were then stably labeled by fluorescent proteins by a lentiviral transduction system. When either edge derived, or core derived subclones were mixed together, growth competition was observed between those subpopulations. In contrast, edge derived subclones were mixed with those from the core derived ones, the relative proportion of each remain unchanged. This data indicate that clonal competition could be dependent on the spatial identity of the original clones. Interestingly, a further shift in the competition was observed when these clones are exposed to temozolomide in the mixed cultures. In addition, the clonal competition was dependent on the ability of proliferation but not on the cell death. Selective apoptosis of non-competing clones was non-significant but were not ruled out. We will further determine the genetic and epigenetic signatures of the dominant and hyper-proliferative subclones in the mixed culture. Currently, in vivo validation of these studies are underway. Our study indicates that understanding spatial heterogeneity at the clonal level will be a crucial step to devise a new strategy for the future treatment.

STEM-18. THE c-Jun N-TERMINAL KINASE (JNK) IS A CRUCIAL COMPONENT OF MAINTENANCE IN GLIOBLASTOMA STEM-LIKE CELLS.

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Glioblastoma (GBM)is the most common malignant primary brain tumors in adults, and despite established therapeutic approaches, the prognosis remains bleak. The cellular heterogeneity within GBM is one of the major causes of poor patient outcomes, whereby GBM cancer stem-like cells (GSC) drive therapeutic resistance. Compelling evidence suggests that GSCs play a major role in initiation, progression, and recurrence of GBM; therefore, there is an urgent need to identify novel therapeutic approaches that targets GSCs. c-Jun N-terminal Kinase (JNK) signaling is chronically altered in several tumors, including GBM. Therefore, defining the functional role of JNK in GSCs is critical to understanding GBM pathophysiology and developing efficacious therapies against this recalcitrant population. In this study, we examined the impact of our potent, brain-penetrant pan-JNK inhibitors (Kamenecka et al., 2010) on cell proliferation, viability, neurosphere formation and migration capacity of GSCs, and compared the novel JNK inhibitors to SP600125, a widely-used JNK inhibitor with poor brain penetrance and selectivity. Our results show that inhibition of JNK using these novel compounds decreased cell viability, proliferation, migration, and neurosphere formation capacity of GSCs to a greater extent than SP600125. The use of pan-JNK inhibitors compared to SP600125 decreased the levels of c-Jun and phospho-c-Jun, a JNK target, in GSCs, illustrating the potency of these novel inhibitors. Interestingly, inhibition of JNK with our novel compounds ultimately induced caspases 3 and 7 indicating that the compounds initiate apoptosis mechanism GSCs. Small molecule inhibitors with good brain penetrance that decrease cell proliferation, migration, and viability in GSCs, offers a novel therapeutic strategy to eliminate GSCs from tumors by primed them for apoptosis. Kamenecka T, et al. (2010) Synthesis, biological evaluation, X-ray structure, and pharmacokinetics of aminopyrimidine c-jun-N-terminal kinase (JNK) inhibitors. J Med Chem 53:419–31.

STEM-19. MONOCARBOXYLATE TRANSPORTER-4 DEPLETION INHIBITS STEMNESS, PROMOTES DNA DAMAGE AND RADIOSENSITIZES GLIOBLASTOMA STEM CELLS

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Glioblastoma multiforme (GBM) is the most aggressive brain tumor in adults, with a median survival for patients of 12–15 months after diagnosis. Standard therapy includes radiation and chemotherapy, with GBM recurrences refractory to second line treatments. Given the lack of therapeutic opportunities in GBM, new treatment strategies are urgently needed. Glioma stem cells (GSCs) have been shown to support GBM maintenance and exhibit enhanced resistance to ionizing radiation, a cornerstone of GBM therapy. Monocarboxylate transporter-4 (MCT4, SLC16A3) is highly expressed in the vast majority of GBMs and predominantly in cells that congregate in "palisades" around centers of necrosis or in cells where hypoxia-inducible factor-1alpha (HIF-1α) is expressed either due to reduced oxygen levels or other microenvironmental stresses. MCT4 appears to regulate proliferation, survival, and xenograft implantation of GSC neurosphere lines. Importantly, we found these effects to be independent of lactate homeostasis. Gene set enrichment analysis (GSEA) of differentially expressed genes revealed an enrichment of DNA replication stress response pathways including cell cycle (G2/M checkpoint), DNA replication, DNA damage response (DDR), and cellular deoxyribonucleotide metabolism in MCT4-depleted GSCs. To validate these data, after lentiviral silencing of MCT4 expression in two GBM neurosphere lines, we examined DNA repair potential by assessing the resolution of γH2AX and by performing alkaline comet assays. In both assays, reduced MCT4 levels resulted in a dramatic increase in the number of nuclear foci ($p < 0.0001$) and in the percentage of cells with comet tails ($p=0.0003$), respectively, confirming that reduced MCT4 expression decreases cellular ability to repair DNA damage. Moreover, we show that conditional MCT4 depletion alone ($p=0.0001$) or in combination with ionizing radiation ($p=0.0006$) efficiently enhance survival of mice orthotopi-

cally implanted with GSC-derived xenografts. We determined that MCT4 inhibition enhances the radiosensitivity of GBM and should be explored as a treatment strategy to complement conventional treatment.

STEM-20. Spt6 REGULATES TRANSCRIPTION BY STABILIZING RNA Pol II AND SO DRIVES GLIOBLASTOMA CANCER STEM-LIKE CELL MAINTENANCE

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Glioblastoma (GBM) is the most advanced and lethal tumor with epigenetically distinct cancer stem-like cells (CSCs) resistant to chemo- and radiotherapy. Spt6 is a histone chaperon involved in nucleosome assembly and regulates RNA polymerase II-mediated transcription. Our preliminary data identified Spt6 to be crucial for glioblastoma stem cells (GSCs) genomic stability and survival. Spt6 depletion in GSCs decreased global transcription rates and cell cycle entry. Cycloheximide and proteasome inhibitor (MG132) experiments confirmed that Spt6 stabilizes RNA polymerase II and so regulates GSC maintenance at a transcriptional level. Spt6 loss impaired GSC survival *in vitro* and tumor initiating capacity *in vivo*, thereby making it an attractive target for glioblastoma therapy.

STEM-21. DECIPHERING THE RESPONSE OF SUBVENTRICULAR ZONE-NESTED GLIOBLASTOMA CELLS AFTER SURGERY

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INTRODUCTION: Glioblastoma (GBM) is the most frequent malignant brain tumor in adults, with poor prognosis, subsequent to systematic recurrences, which occur in 80% of cases in the resection margin of initial tumor. We previously demonstrated that, after experimental striatal xenotransplantation, GBM cells, and particularly GBM-initiating cells (GIC), are able to escape the tumor mass and specifically colonize the sub-ventricular zone (SVZ), a well-known neurogenic zone in adult brains. We also demonstrated that this specific oriented migration is driven by a CXCL12-CXCR4 signalization. In this study, we address the potential implication of SVZ-nested tumor cells in local GBM relapses. MATERIALS AND METHODS: We engrafted in the right striatum of nude mice GBM cells (GB138) from a human primary culture, which are previously transfected with a lentiviral construction in order to express the RFP spontaneously, while they conditionally express eGFP, only in presence of Cre-recombinase. As the GB138 cells reach the SVZ, we injected in the lateral ventricle an Adeno-Associated Viral vector expressing Cre-recombinase, which is able to infect nearby the SVZ cells, including the GB138 cells that have reached this region. Finally, we compared 3 mice that were not operated (control group) with 3 mice that underwent tumor resection (surgery group) and quantify green spots in the tumor mass (TM) and/or resection cavity (RC) every 20 microns thanks to Lightsheet microscope after tissue clarification. RESULTS: We found that the median of green spots for the 3 mice of the control group was respectively 1, 0 and 0 in TM, while the median for the surgery group was 7, 8 and 47 in RC. The Standard Deviation (SD) for the control group was respectively 1.1, 0.4 and 0.6, while SD for the surgery group was respectively 1.5, 1.7 and 16. CONCLUSION: SVZ-nested GBM cells seem to be recruited for tumor relapse after surgery.

STEM-22. UPTAKE OF P-BORONO-PHENYLALANINE BY BRAIN TUMOR STEM LIKE CELLS ANALYZED BY MASS CYTOMETRY

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INTRODUCTION: Because of chemo- and radio-resistance of brain tumor stem cells, they could be the origins of recurrent malignant glioma. We have used boron neutron capture therapy (BNCT) to treat patients with either recurrent or newly diagnosed malignant glioma resulting in a significant increase in median survival of patients. BNCT is a form of tumor-selective particle radiation therapy consisting of two components. First, a boron-10 (^{10}B)-containing drug is administered, followed by irradiation with epithermal neutrons. The resulting $^{10}B(n,\alpha)^{7}Li$ capture reaction produces alpha particles whose short path length (5–9 μm) results in the selective killing of tumor cells with a concomitant sparing of adjacent normal tissues. *P*-boronophenylalanine (BPA) is a chemical compound used in clinical trials in BNCT. BPA accumulates preferentially in growing cells rather than in quiescent cells of the tumor. Here, we investigate whether brain tumor stem like cells take up BPA or not using mass cytometry (Cytof). METHODS: We used brain tumor stem like cells (SLC) and the cells differentiated by fetal bovine serum from them (DC). After exposure to BPA for 24 hours at the concentration of 25ppm in 5% CO_2 incubator, we immune-stained them using twenty stem cell markers, anti-Ki-67, anti-BPA and anti-CD98 (heterodimer that forms the large neutral amino acid transporter to take up BPA) antibody and analyzed with Cytof. RESULTS: Two to three times larger number of SLC were BPA or CD98 (+) than DC. In BPA or CD98 (+) cells in SLC, although the number of Ki-67 (+) cells were only two to three times larger than negative cells, that of stem cell marker (Oct3, Nestin, Sox2, and PDGFRa) (+) cells were two to six times larger than negative cells CONCLUSION: Stemness may influence the uptake of BPA. In following experiments, effect of tumor micro-environment on BPA uptake is to be cleared.

STEM-23. miR-486-5p REGULATES TUMOR SUPPRESSOR NETWORKS AND ITS INHIBITION REDUCES TUMOR VOLUME AND SENSITIZES TO RADIATION TREATMENT IN A PDX MOUSE MODEL OF GBM

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Glioblastoma multiforme (GBM) is a heterogeneous malignancy that contains a subpopulation of tumor cells with stem-like features. These GBM stem cells (GSCs) have the ability to self-renew and establish tumors. As such, they are thought to disproportionately contribute to re-growth and resistance to therapy, and targeting mechanisms that underlie stemness may lead to development of more effective treatments. Work from the Laterra lab has shown that the transcription factors (TFs) Oct4 and Sox2 drive GBM cells toward a stem-like phenotype, in part by regulating subsets of microRNAs (miRNAs). One commonly overlooked mechanism by which these TFs can promote tumorigenesis is by inhibiting repressors of cell stemness and tumor propagation through miRNAs. This study demonstrates that miR-486-5p is induced by Sox2 12-fold and regulates GSC self-renewal as measured by limiting dilution and sphere formation assays by inhibiting tumor suppressors PTEN and FOXO1 in established glioma cell lines and patient-derived neurospheres. Inhibition of endogenous miR-486-5p in these lines leads to cell death. Furthermore, *in vivo* delivery of miR-486-5p inhibitor via a novel nanoparticle complex to pre-established patient-derived GBM xenografts (PDX) in mice decreases tumor volume by 61% ($p < 0.001$) and sensitizes to the effects of ionizing radiation 9-fold ($p < 0.001$). Thus, the Sox2:miR-486-5p axis offers a promising translational target for therapies aimed at depleting GBMs of stem-like cells in combination with standard therapies.

STEM-24. IDENTIFICATION OF SERPIN B3 AS A JUNCTIONAL ADHESION MOLECULE A BINDING PARTNER IN GLIOBLASTOMA CANCER STEM CELLS

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Cancer stem cells (CSCs) in glioblastoma (GBM), the most lethal primary brain tumor, contribute to tumor progression and therapeutic resistance. While we previously demonstrated that junctional adhesion molecule A (JAM-A), a cell adhesion molecule, is necessary and sufficient for CSC maintenance, the signaling networks underlying this function have yet to be elucidated. JAM-A has several known binding partners in endothelial cells. To identify GBM CSC-specific binding partners, we introduced JAM-A into CSCs, conducted a pull-down assay, and determined binding partners via mass spectrometry. The top candidate identified was Serpin B3, a serine/cysteine proteinase inhibitor and key regulator of epithelial to mesenchymal transition. We validated the endogenous interaction between JAM-A and Serpin B3 via co-immunoprecipitation and confirmed binding dynamics in vitro via surface plasmon resonance. Like JAM-A, Serpin B3 was enriched in CSC patient-derived xenograft models. Immunostaining revealed colocalization of Serpin B3 and JAM-A, in vitro and in vivo. Overexpression of Serpin B3 was found to increase stem cell marker expression. Ongoing studies are assessing proliferation, self-renewal, and tumor initiation in the context of Serpin B3 overexpression in non-CSCs and Serpin B3 knockdown in CSCs. Additional mechanistic studies using JAM-A mutant constructs will determine the precise binding site between JAM-A and Serpin B3 and serve as the basis for developing therapeutics that inhibit the interaction between JAM-A and Serpin B3. Our findings provide a novel JAM-A binding partner and reveal a putative interaction that may be disrupted to compromise CSC maintenance.

STEM-25. SINGLE-CELL SIGNATURES UNCOVER GLIAL PROGENITOR HETEROGENEITY AND MOLECULAR DETERMINANTS FOR GLIOMA GROWTH

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The major glial subtypes in the brain, oligodendrocytes and astrocytes, are heterogeneous populations, however, their progenitor diversity and contribution to malignant transformation remain elusive. Despite recent progress in the evaluation of oligodendrocyte and astrocyte heterogeneity in adult brain regions, the diversity and molecular profiles of their progenitors remains incompletely understood. In addition, a comprehensive understanding of transcriptional dynamics of glial progenitors and their lineage determinants underlying normal development and malignant transformation is currently lacking. To address the heterogeneity of glial progenitors, we performed targeted high-throughput single-cell RNA sequencing of prospective astrocyte lineage cells and oligodendrocyte precursor populations isolated by fluorescence activated cell sorting from the neonatal cortices and mapped single-cell expression profiles and molecular features of glial subtype progenitors. We found that astrocyte lineage cells are much more dynamic than previously appreciated and exhibit distinct lineage developmental trajectories in the developing neonatal cortex. In contrast to the astrocyte lineage, the progenitors of oligodendrocytes (OPC) exhibited a cellular continuum, which included a previously unrecognized primitive OPC subpopulation prior to the committed OPCs. Application of scRNA-seq to a murine model of malignant glioma revealed cycling OPC serving as a specific cellular niche contribute to glioma formation. We also established a new algorithm to identify transcription-factor-driven networks and discovered a set of regulators critical for controlling glial lineage specification and glioma growth. Thus, our single-cell analyses reveal distinct dynamics and heterogeneity of glial progenitors during brain development. Moreover, we identify analogous glial progenitors as the important source for glioma growth, and further define a molecular link between gliogenesis and gliomagenesis, thereby pointing to potential cellular and molecular targets for glioma treatment.

STEM-26. MICROENVIRONMENTAL FGF2 INDUCES GLIOBLASTOMA STEM CELLS THROUGH THE FGFR1-ZEB1 AXIS

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Glioblastoma is the most lethal and aggressive brain cancer in adults. Poor prognosis is due to resilience to therapy and tumour recurrence, which have been linked to glioblastoma stem-like cells (GSCs). FGF2 and its cognate receptors have been linked to malignancy and progression in glioma, and FGF2 is frequently employed in GSC culture paradigms. The specific mechanisms of how this growth factor promotes stemness and malignancy in glioblastoma remain incompletely understood. Therefore, we analysed expression of FGF receptors (FGFRs) and the effects of FGF2 on patient-derived glioblastoma cell lines. We found that FGF2 induces expression of the stemness-associated transcription factor ZEB1, increases sphere formation frequency and cell migration. Analysis of FGF receptor expression and function using knockdown approaches in patient-derived glioblastoma cell lines revealed that FGFR1 is relevant for stem cell maintenance. FGFR1 knockdown reduces sphere and colony formation, and increases survival in a xenograft mouse model. Analysis of large-scale gene-expression datasets revealed association of FGFR1 in mesenchymal glioblastoma, and increased FGFR1 expression in 30–40% of cases. We identify FGFR1 as a potential GSC marker and therapeutic target.

STEM-27. THE ALTERATION OF IMMUNOSUPPRESSIVE FUNCTION IN GLIOBLASTOMA WITH UNDIFFERENTIATED TRANSFORMATION

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Glioma stem-like cells (GSCs) are strongly related to the treatment resistance in glioblastoma (GBM). GSCs differentiate into differentiated glioma cells (non-GSCs), and lose the stem cell features such as self-renewal, pluripotency and tumorigenesis. However, several study have demonstrated that GSCs and non-GSCs could convert between each other. Recently, immunotherapy has been attracting attention as a new GBM therapy. Indoleamine 2,3-dioxygenase (IDO), the enzyme for Tryptophan metabolism, is involved in the ability of GBM to escape from immune surveillance, and show immunosuppressive function. In this study, we investigated the difference of IDO expression between GSCs and non-GSCs to search a new therapeutic target for GBM. We used the human malignant glioma cell lines U-251MG and Rev-U-251MG, which was established by culturing U-251MG cells in serum-free media. Rev-U-251MG formed spheres with increased level of Nestin and Nanog expression. We regarded Rev-U-251MG as a GSCs cell line model and investigated the expression of IDO. The expression levels of IDO mRNA and protein were increased in Rev-U-251MG compared to the expression in U-251MG. The expression levels of *IDO1* mRNA were analyzed by the quantitative reverse transcription PCR ($p<0.01$, n=6), and IDO1 protein were analyzed by Western blotting. These results suggest that the GSCs strongly escape from immune surveillance while producing more IDO compared to non-GSCs. It is important to know about the alteration of immunosuppressive function between GSCs and non-GSCs to establish a new treatment strategy for GBM. The possibility was suggested that GSCs immunosuppressive function via expression of IDO could be a new target for the GBM therapy.

STEM-28. TISSUE FACTOR PROMOTES THE GLIOMA STEM CELL PHENOTYPE, AND IS SUPPRESSED BY MUTANT IDH1

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Isocitrate dehydrogenase 1 mutant ($IDH1^{mut}$) gliomas have global genomic hypermethylation, are less aggressive than IDH1 wild-type ($IDH1^{wt}$) gliomas, and generally grow poorly *in vitro* and *in vivo*. Yet little data exist that connect specific hypermethylation targets to this unique phenotype. We previously reported that the gene encoding Tissue Factor (TF), *F3*, which promotes both thrombosis and malignant behavior, is among the most hypermethylated and downregulated genes in $IDH1^{mut}$ gliomas. In multiple $IDH1^{wt}$ and $IDH1^{mut}$ patient-derived glioma cell lines, *F3* was hypermethylated in $IDH1^{mut}$ cells compared to $IDH1^{wt}$ cells, with reduced TF protein expression. A demethylating agent, decitabine, increased *F3* transcription in $IDH1^{mut}$ glioma cells, but not in $IDH1^{wt}$ cells. TF knockdown greatly reduced proliferation, colony formation, glioma stem cell (GSC) marker expression, and xenograft growth of $IDH1^{wt}$/$EGFRvIII^{amp}$ GBM6 cells and $IDH1^{wt}$/$EGFR^{amp}$ GBM12 cells, but not of *NF1*-mutant GBM43 cells. Conversely, TF induction enhanced the proliferation and colony formation of $IDH1^{mut}$ GBM164 and TB09 cells, especially GBM164. TF also increased the *in vivo* "take rate" of intracranial GBM164 xenografts from 0% to 100%, but did not enable TB09 xenograft growth. TF activated receptor tyrosine kinases (RTKs) in GBM6, GBM12, and GBM164, but RTK expression was very low in GBM43 and TB09. Transcriptomic profiling showed that only two genes were downregulated after TF knockdown in GBM6 and GBM12, and also upregulated after TF induction in GBM164: *PROM1*, encoding CD133, and *CTNND2*, encoding δ-catenin. Neither gene was affected by TF manipulation in GBM43 or TB09. High *F3* mRNA correlated with enrichment of GSC markers, and worse outcome, in TCGA gliomas. These data suggest that: (i) TF promotes a GSC phenotype through RTKs; (ii) CD133 and δ-catenin may be critical effectors of TF-induced GSC behavior; (iii) TF methylation reduces $IDH1^{mut}$ glioma malignancy; (iv) TF is an attractive, novel therapeutic target in $IDH1^{wt}$ gliomas.

STEM-29. UNSATURATED FATTY ACID (UFA) METABOLISM REGULATES MEMBRANE-ENDOLYSOSOME-NUCLEAR INTER-ORGANELLE COMMUNICATION IN GLIOMA STEM CELLS

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Stem cells receive the signals from their niches that instruct them to self-renew and prevent them from differentiating through a cascade of inter-organelle communication processes. However, how the inter-organelle communication mechanism functions in the maintenance of stemness in glioma stem cells (GSCs) remains unclear. To identify potential glioma suppressors that affect the interaction of GSCs with their niches, we discovered that the RNA-binding protein Quaking (QKI) is a key regulator of cellular endocytosis. *QKI* is mutated or deleted in ~34% of human glioblastomas. Consistently, 92% of the *Nestin-CreERT2;*$Qki^{L/L}$*;*$Pten^{L/L}$*;*$p53^{L/L}$ (QPP) mice developed glioblastoma with a median survival of 105 days, yet the *Nestin-CreERT2;PtenL/L;p53L/L* mice did not develop any gliomas. Mechanistically, QKI regulates the RNA stability and alternative splicing of numerous protein and lipid components of endolysosomes, particularly the unsaturated fatty acids (UFAs). Notably, lower levels of QKI, endolysosomes, and stearoyl-CoA desaturase (SCD, the key enzyme for UFA biosynthesis) all correlate with poorer

prognosis in glioblastoma patients. Functionally, *Qki* and UFA loss both decrease endolysosome-mediated receptor degradation, thereby enriching receptors on the cytoplasmic membrane (e.g., Frizzled and Notch1) that are essential for maintaining stemness. This enrichment of receptor signaling enables GSCs to cope with the low ligand levels outside their niches. On the other hand, lower lysosomal activity induced by *Qki* and/or UFA loss also lead to defective mitophagy, which consequently leads to accumulation of damaged mitochondria, high level of ROS, and genomic instability in Qki-deficient NSCs. We identified that genomic instability induced by *Qki* deletion led to copy number gains of classical glioblastoma-associated oncogenes such as of PDGFRa and Cyclin D1/D3. Lastly, the heterogeneity of *Nestin-CreERT2;QPP* tumors also lead to heterogeneous responses to immunocheckpoint blockade inhibitors including anti-CTLA4 and anti-PD1. Taken together, our data suggest that Qki/UFA loss-induced endolysosomal defects promotes gliomagenesis through both reducing receptor degradation and inducing genomic instability.

SURGICAL THERAPY

SURG-01. AN INTRAOPERATIVE RAMAN SPECTROSCOPIC PROBE FOR GLIOMA SURGERY: INDICATIONS, SAFETY, AND FUTURE DIRECTIONS

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INTRODUCTION: Raman spectroscopy is a tool that utilizes a non-contact label-free modality of optical imaging that measures inelastic scattered photon shifts to give a unique biochemical signature. There is established work showing that these unique fingerprints can differentiate a glioma from necrosis and normal brain. The ability to instantly characterize these organic tissues intraoperatively, then, can help guide a surgeon's resection. The goal of the present study is to review the Raman work that has been performed to date, its demonstrated safety in a rat model and the indications for use of this operative tool. METHODS: We review the fundamental principles of Raman spectroscopy, some contemporary data, and show its effectiveness as an intraoperative probe. Because the probe uses a 100mW 785nM laser wavelength, we also determined the threshold at which it causes damage to cortical grey matter in a rat model. RESULTS: Raman spectroscopy has a high sensitivity and specificity (>97%) when discriminating between grey matter, necrosis and GBM. A discriminant function analysis, supervised classification algorithm, is used for spectral identification to allow relevant spectral interpretation and allows for tailoring to which Raman peaks are significant for diagnosis. In regards to safety, animal experiments showed no damage seen at 10 seconds/250mW and estimated damage to occur at 60 seconds/250mW. We also present an intraoperative workflow of how this tool could be used in the operating room. CONCLUSION: A Raman spectrographic probe is a safe and powerful tool that can provide live intraoperative diagnosis and identify tissue margins. Further work is being done to identify clinical outcomes, the unique fingerprints of other pathological tissues and further differentiate gliomas based on molecular markers like IDH-1.

SURG-02. A NOVEL RISK MODEL TO DEFINE THE RELATIVE BENEFIT OF MAXIMAL EXTENT OF RESECTION WITHIN PROGNOSTIC GROUPS IN NEWLY DIAGNOSED GLIOBLASTOMA

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Although the overall prognostic significance of maximal surgical resection of contrast-enhancing tumor in glioblastoma patients is well established, prior studies have not evaluated the combined importance of resection, molecular markers, patient characteristics, and chemoradiation. Incorporation of these factors may redefine the relative benefit of cytoreductive surgery and establish differing thresholds for extent of resection in varying clinical presentations. In the first study of its kind, we examine the interactive effects of volumetric extent of resection with molecular and clinical factors to develop a new roadmap for cytoreductive surgery. Based on a 20-year retrospective cohort of 850 glioblastoma patients who had initial surgery at UCSF, we employed survival models and recursive partitioning (RPA) to investigate multivariate relationships of overall survival (OS), both in the entire cohort as well as a subset diagnosed since 2005 (Stupp-era) with IDH1 mutation status available (n=470). For the entire cohort and the Stupp-era subset, the RPAs elucidate the combinatorial consequence of treatment, age, IDH1 status (in the subset), and resection of both enhancing and non-enhancing tumor. In the Stupp-era, temozolomide-treated patients that are IDH-wildtype and >65 clearly benefit from a reduction of the enhancing tumor (median OS: 10.1 vs 15.8 months). IDH-wildtype, temozolomide-treated patients under 65 benefit from reduction of both enhancing and non-enhancing tumor with a median survival similar to that of IDH-mutant, temozolomide-treated patients (combined median OS: 33.7 months). The patients faring worst are those that did not receive temozolomide that are >65 and/or have ≥0.3 cm^3 residual enhancing tumor (median OS: 4 months). These risk models outperform all published prognostic models. This is the first study to combine resection of contrast-enhancing and non-enhancing tumor in conjunction with molecular and clinical information in a large single-institution study, and paves the way for rethinking surgical strategies for individual patients with newly diagnosed glioblastoma.

SURG-03. A COMPARISON OF SURVIVAL OUTCOMES AFTER BIOPSY VERSUS RESECTION IN PRIMARY CNS LYMPHOMA: A SINGLE INSTITUTION EXPERIENCE

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INTRODUCTION: Primary CNS lymphoma (PCNSL) is an aggressive, often multifocal neoplasm sensitive to chemoradiation. Surgery has conventionally been diagnostic biopsy rather than resection. Recently, studies have challenged this paradigm, suggesting resection is safe and possibly more efficacious. We addressed this via analysis of our institutions experience. METHODS: A retrospective review was conducted in patients treated with surgery and chemotherapy with or without radiation for PCNSL between March 2002 and February 2018. Indications for surgery were predominantly for tissue diagnosis. Statistical analyses included Kaplan-Meier, log-rank, and Pearsons chi-squared analyses. RESULTS: There were 138 patients (mean age, 61.2-years; range, 14.9–89.7). 5 had GTR, 13 had STR, and 120 had biopsy. Biopsied patients (45/75, 37.5%) harbored more multifocal lesions than GTR (0/5, 0%) and STR (2/11, 15.4%) (p=0.03). Complete remission rates at 6-months were similar in biopsy (67/120, 55.8%) vs GTR (2/5, 40%) or STR (7/13, 53.8%) (p=0.78), even when GTR and STR were combined (p=0.64). There were no differences in PFS between GTR and STR: 8.5-months [95%-CI: 0.2–16.9] vs 21.3-months [4.6–38.0], respectively (p=0.22) or OS: 37.8-months [0–100.1] vs 28.3-months [10.7–45.8], respectively (p=0.96). For biopsy, PFS: 26.5-months [19.9–33.1] and OS: 39-months [30.8–47.2] did not differ from GTR (PFS: p=0.14, OS: p=0.87) or STR (PFS: p=0.83, OS: p=0.30) (Fig. 1). Likewise, when data from GTR and STR were combined (PFS: 17.7-months [0–38.1)], OS: 30.9-months [10.6–51.3]), there remained no significant differences compared to biopsy (PFS: p=0.43) (OS: p=0.50). 7/11 resected patients (6 STR, 1 GTR) had improvement in preoperative deficits after resection, including one with rapid relief of life-threatening mass effect. CONCLUSION: Our experience supports the current surgical paradigm towards PCNSL, demonstrating resection does not improve survival outcomes over biopsy. Resection, regardless of GTR or STR, may have a role in rapid relief of preoperative symptoms, but further studies are needed to answer this question.

SURG-04. SURVIVAL BENEFIT ASSOCIATED WITH GROSS TOTAL RESECTION IN GRADE II ASTROCYTOMAS: AN INTEGRATED ANALYSIS OF THE SEER AND TCGA DATABASE

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We used the Surveillance, Epidemiology, and End Results (SEER) to determine the survival benefit associated with gross-total resection (GTR) of grade II diffuse astrocytoma (DA) stratified by age and tumor location. We further correlated these findings to the prevalence of isocitrate dehydrogenase mutations (mIDH) in similarly age- and location-stratified DA patients from The Cancer Genome Atlas (TCGA). The SEER database was used to assess GTR associated survival benefits relative to subtotal resection (STR), and TCGA database was used to determine the prevalence of IDH mutation. Survival analysis was accomplished using Kaplan-Meier curves, log-rank tests, and multivariable Cox proportional hazards models. GTR was associated with significant survival benefit for all DA patients with frontal tumors irrespective of age (age < 50, HR 0.56, p=0.002; age > 50, HR 0.41, p< 0.001). However, in patients with non-frontal tumors, only those < 50 derived a survival benefit from GTR (< 50 HR 0.55, 95%, p=0.002; > 50 HR 0.78, 95% p=0.114). This pattern of GTR-associated survival in DA patients differed significantly from our previous finding in AA patients, where significant survival benefit was only observed in patients < 50 with frontal tumors who underwent GTR. While the prevalence of mIDH status in the TCGA DA and AA's generally tracked with the above described survival patterns, notable exceptions were observed. For instance, while the prevalence of mIDH in patients age > 50 with non-frontal tumor were comparable for DA and AAs (82% versus 75%), the GTR associated survival benefit in these patients differed significantly. Our results suggest that the survival benefit of GTR, prevalence of mIDH, and relationship of these variables to patient age and tumor location differs between DA and AA. While mIDH status generally correlated with survival patterns, instances where these patterns differed suggests complex interactions between age, location, mIDH status and tumor grade as prognostic factors.

SURG-05. NAVIGATED INTRA-OPERATIVE 2-D ULTRASOUND VS STANDARD NEURONAVIGATION IN HIGH GRADE GLIOMA SURGERY

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Maximizing extent of resection (EOR) and reducing residual tumor volume (RTV) while preserving neurological functions is the main goal in the surgical treatment of gliomas. Navigated Intra-operative ultrasound (N-ioUS) is a real-time imaging technique which, combining the advantages of ultrasound and conventional neuronavigation (NN), allows for overcoming the limitations of the latter. We evaluate the impact of real-time NN combining ioUS and pre-operative magnetic resonance imaging (MRI) on maximizing EOR in glioma surgery compared to standard NN. We retrospectively reviewed a series of 60 cases operated on for supratentorial gliomas, 31 operated under the guidance of N-ioUS and 29 resected with standard NN. Age, location of the tumor, pre- and post-operative Karnofsky Performance Status (KPS), EOR and, if any, post-operative complications were evaluated. Volumetric pre-operative and 48hours post-operative MRI was used to determine EOR. The rate of gross total resection (GTR) in NN group was 44.8% and EOR≤90% 10.3%, whereas in N-ioUS group a 61.2% GTR rate was obtained with a 6.4% rate of EOR≤90%. The rate of RTV> 1cc for GBMs was significantly lower for the N-ioUS group (p=0.01) compared to the NN. In 13/31 (42%) RTV was detected at the end of surgery with N-ioUS. In 8 of 13 cases (25.8% of the cohort) surgeons continued with the operation until complete resection. Specificity was greater in N-ioUS (42% vs 31%) and positive predictive value (73% vs 54%). At discharge the difference between pre and post-operative KPS was significantly higher for the N-ioUS (p=0.0008). Using N-ioUS-based real-time guidance in glioma surgery we obtained superior results in terms of both EOR and neurological outcome, in comparison to standard NN. N-ioUS has proven usefulness in detecting RTV> 1cc. In tumors located nearby eloquent areas the technique was successfully combined with cortical and subcortical mapping techniques.

SURG-06. LASER ABLATION FOR BRAIN METASTASES: SAFETY AND PRELIMINARY OUTCOMES FROM THE LASER ABLATION OF ABNORMAL NEUROLOGICAL TISSUE USING ROBOTIC NEUROBLATE SYSTEM (LAANTERN) REGISTRY

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INTRODUCTION: Laser Interstitial Thermal Therapy (LITT) is a novel technology that offers a minimally invasive option for brain metastases. The current understanding of LITT in this context is limited by small sample size or to single center experiences. Laser Ablation of Abnormal Neurological Tissue using Robotic NeuroBlate System (LAANTERN) is a registry designed to address these limitations. We present the preliminary experience with regard to safety, procedural data, and preliminary outcomes of LITT for brain metastases. METHODS: LAANTERN is an ongoing registry that collects data on patients treated with the NeuroBlate® system. Data presented here include procedural data, complications, and survival. RESULTS: Fifty-one brain metastases in 50 patients (33 male, 17 female) were treated with LITT. Mean age was 58.3 ± 13.4 years (range 25–80 years). 48 patients (96%) had prior treatment(s) to the target lesion, including surgery, chemotherapy, and radiation. 49 lesions (96.1%) were treated with a single trajectory, and 2 (3.9%) were treated with two trajectories. Location of lesions were frontal lobe (26, 51%), followed by parietal (10, 19.6%) and temporal (9, 17.6%) lobes. Average lesion volume was 2.9 ± 3.2cm3. Volumetric data was available for 48 patients; of these, 40 (83%) had 91–100% ablation coverage based on physician determination; eight (16.7%) had 51–90% ablation coverage. The 91–100% group had a death rate of 18.9%; the 51–90% group had a death rate of 37.5% (p=0.35). Adverse events occurred in 5 (10%) patients within 30 days of procedure. There were no mortalities in this timeframe. Median follow up was 6 months; median overall survival has not yet been reached. Kaplan-Meier survival estimates at 1, 6, and 12 months were 90%, 76%, and 71%, respectively. CONCLUSION: Preliminary results from the LAANTERN registry demonstrate an acceptable safety profile, procedural efficacy, and satisfactory outcomes of LITT in carefully selected patients with brain metastases.

SURG-07. BETWEEN-HOSPITAL VARIATION IN MORTALITY AND SURVIVAL AFTER GLIOBLASTOMA SURGERY

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PURPOSE: To measure between-hospital variation in risk-standardized survival outcome after glioblastoma surgery and to explore the association between survival and hospital characteristics in conjunction with patient-related risk factors. METHODS: Data of 2,409 adults with first-time glioblastoma surgery at 14 hospitals were obtained from a comprehensive, prospective population-based Quality Registry for Neurological Surgery in the Netherlands between 2011 and 2014. We compared the observed survival with patient-specific risk-standardized expected early (30-day) mortality and expected late (2-year) survival, based on patient age, performance status, and year of treatment. Summarized outcomes per hospital were analyzed in funnel plots. Hospital characteristics were analyzed in logistic regression and Cox proportional hazards mod-

els. RESULTS: Overall 30-day mortality was 5.2% and overall 2-year survival was 13.5%. Median overall survival varied between 4.8 and 14.9 months among hospitals, and biopsy percentages ranged between 16% and 73%. One hospital had lower than expected early mortality, and four hospitals had lower than expected late survival. Higher hospital volume was related with lower early mortality (P=0.031). A 10% increase in volume was associated with 3.9% relative decrease in early mortality, but not with overall survival. Patient-related risk factors (lower age; better performance; more recent years of treatment) were significantly associated with longer overall survival. Of the hospital characteristics, longer overall survival was associated with lower biopsy percentage (HR: 2.09, 1.34–3.26, P=0.001), and not with academic setting (HR: 0.951, 0.858–1.05), nor with hospital volume (HR: 0.954, 0.866–1.05). CONCLUSION: Hospitals vary more in late survival than early mortality after glioblastoma surgery. Widely varying biopsy percentages indicate treatment variation. Patient-related factors have a stronger association with overall survival than hospital-related factors.

SURG-08. SURVIVAL BENEFIT OF LOBECTOMY FOR PRIMARY GLIOBLASTOMAS IN NON-ELOQUENT REGION: GROSS-TOTAL RESECTION VERSUS SUPRATOTAL RESECTION

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OBJECTIVE: This single center retrospective study aims to assess survival benefit of lobectomy compared to gross-total resection without lobectomy for primary glioblastomas (pGBM) in non-eloquent region. BACKGROUND: The only prognostic factor of glioblastoma that can be determined by the surgeon is the extent of resection. However, glioblastomas are known to infiltrate to a wider extent and are eventually recurred by remaining microscopic tumors. In surgical oncology field, the classic concept of radical resection was wide resection including normal surrounding tissue. Nevertheless, it is not yet known whether further removal of tumor-surrounding tissue may be helpful for survival in pGBM patients. METHODS: Among the patients who had undergone surgical resection and were diagnosed as glioblastoma by histopathologic examination, we selected patients who had complete resection with the lesion localized on the non-dominant frontal or temporal lobe. Patients were divided into two groups: those who underwent gross-total resection of the tumor without additional tissue resection (GTR group) and those who underwent gross-total resection of the tumor with additional lobectomy (SupTR group). Progression-free survival (PFS), overall survival (OS) and postoperative Karnofsky performance scale (KPS) score were compared between groups. RESULTS: Thirteen of 28 patients underwent complete resection only, and 15 patients underwent complete resection with additional frontal or temporal lobectomy. The median PFS time after surgery was 13.1 months (95% CI 6.4–19.8) in GTR group and 38.3 months (95% CI 11.9–64.7) in SupTR group (p=0.098), respectively. The median OS time after surgery was 14.4 months (95% CI 12.1–16.7) in GTR group and 35.0 months (95% CI 17.4–52.6) in SupTR group (p=0.018), respectively. The mean postoperative KPS score was 73.4 in GTR group and 71.33 in SupTR group (p=0.586), respectively. CONCLUSIONS: In the non-dominant frontal or temporal glioblastomas which were completely resectable, additional lobectomy improved overall survival, without deteriorating performance of the patients.

SURG-09. AWAKE CRANIOTOMY FOR BRAIN TUMOR IN OCTOGENARIANS AND NONAGENARIANS

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INTRODUCTION: Awake craniotomy allows for maximal tumor resection while improving survival and preserving functional status. This procedure is well tolerated in the elderly population (age 65–80). We report the Ottawa Hospital experience with patients 80 years old. METHODS: A chart review (research ethics board approved) of awake craniotomies performed between July 2009 and February 2018 was completed. 14 patients 80 years old at the time of surgery were identified, with ages ranging from 80–93, averaging 83 ± 3.6 years old. Preoperatively, 12 patients had Karnofsky Performance Status (KPS) 80, 2 patients KPS 70. 8 tumors were frontal, 4 temporal, and 2 parietal. Average tumor volume was 23.9 ± 17.9 ml. Pathologies revealed 8 high grade gliomas, 4 metastatic lesions, 1 atypical meningioma and 1 case of radiation necrosis. RESULTS: Intraoperative functional mapping was successful in all the cases. Neuroanesthesiologists followed the protocol involving continuous infusion of low-ultra low dose of propofol, remifentanil and ketamine. Surgical time was 335 ± 84 min. Gross total resection was achieved in 8 patients; near total resection in 6 patients as confirmed by postoperative MRI. Ten patients (62%) had very good outcome with improvement or similar, as before surgery functional status at the time of discharge home, which averaged 4.7 ± 2 days post-operatively. Four patients (38%) had prolonged hospital stay of 37 ± 37 days post-operatively. Two of those patients had low KPS pre and post-operatively. The other two patients had transient increase in neurological deficit postoperatively requiring hospitalization. After initial improvement their functional status deteriorated post radiation therapy, which was started 1 month after surgery. There was no mortality over 30 days postoperatively. CONCLUSIONS: Octogenarians and nonagenarians tolerate surgery well following Ottawa Awake Craniotomy Protocol, especially when their preoperative functional status is good. Early good postoperative outcomes may be affected by their ensuing radiation therapy.

SURG-10. MICROSURGERY OF LIMBIC AND PARALIMBIC GLIOMAS: EXTENT OF RESECTION, MORBIDITY AND SURVIVAL

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OBJECT: To describe the authors experience with microsurgical resection of limbic and paralimbic gliomas by retrospectively analyzing surgical results, neurological and survival outcomes. METHODS: Adults who had undergone resections of limbic and paralimbic gliomas of all grades were included. Tumor location was differentiated according to the Yasargil classification. The extent of resection (EOR) was assessed using a quantitative, semiautomated volumetric analysis of pre and postoperative MR images. RESULTS: Fifty-six consecutive patients, with a median age of 48.8 years, a Karnofsky Performance Score (KPS) of 90 and a median tumor volume of 38.9 cm3 were followed for 2.9 years (median). The majority of gliomas involved combinations of the frontoorbital-insula-mediobasal temporal areas (53%, Type 5), followed by the mediobasal temporal (25%, Type 1), insular region (18%, Type 3), and cingulate gyrus (14%, Type 2). An EOR> 90% was achieved in 55% of the procedures with a median EOR of 91.8%. Permanent disabling motor and language deficits were recorded in 3.6% and 1.8% of patients. The 5-year overall survival (OS) was 90% for Grade II gliomas. Median OS was 15 months and 14 months for Grade III and IV gliomas. A multivariate analysis of various prognostic variables (age, preoperative KPS score, preoperative tumour volume, EOR, residual tumour volumes,IDH1/IDH2 status, 1p19q status, MGMT methylation, postoperative chemotherapy and radiation therapy) showed that WHO grade, poor preoperative KPS decreased postoperative KPS performance predicted survival and disease progression CONCLUSIONS: Extensive resection is often achievable and is relatively safe even in patients with limbic and paralimbic gliomas of all grades. The prognosis of these tumor may not differ substantially from those of hemispheric gliomas located elsewhere and microsurgical resection may not substantially influence their clinical course. It remains unclear, which subset of patient or tumor characteristics may benefit more and which EOR cut-off provides the optimal benefit.

SURG-11. PATHOLOGICAL INVESTIGATION OF NOVEL SPRAY-TYPE FLUORESCENT PROBES FOR BRAIN TUMORS

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OBJECTIVE: A fluorescent probe such as 5-aminolevulinic acid achieves greater extent of resection in glioma surgery and thus serves as a valuable surgical adjunct. However, its sensitivity and specificity are yet to be perfectly satisfactory. Our novel spray-type fluorescence system using green hydroxymethyl rhodamine green (HMRG) probes has been tested for brain tumors. In this study, we investigated on red dimethyl silicone rhodamine 600 (2MeSiR600) probes, which have a higher sensitivity for peptidase activity than HMRG probes. MATERIALS AND METHODS: Fresh tumor tissues as well as fresh frozen tissues harvested from gliomas resected at our institutions were used in this study. The probe library included over 400 types with different combinations of amino acids attached to 2MeSiR600. Fluorescence intensity was measured over

time after probes were applied to homogenized lysates made from fresh frozen tissues. Fluorescent probes which clearly discerned the tumor area from the peripheral area were selected, and then applied to fresh tumor tissues to confirm their selectivity. Tumor tissues were fixed with formalin and stained with hematoxylin and eosin. Immunohistochemistry staining was also performed. The correlation between fluorescence intensity and histopathology including cell density and MIB-1 labeling index was investigated. RESULTS: Probe screening demonstrated some 2MeSiR600 probes exhibited significantly higher fluorescence intensity in the peripheral area compared to the tumor area, which was confirmed when they were applied to fresh tumor tissues. CONCLUSION: 2MeSiR600 probes which selectively fluoresce the peripheral area may enable highly selective tumor recognition in conjunction with 5-aminolevulinic acid or our HMRG probes.

SURG-12. ANTERIOR SKULL BASE TUMOR RESECTION BY TRANSCILIARY SUPRAORBITAL KEYHOLE CRANIOTOMY

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The prognosis and recurrence rate after resection of an anterior skull base lesion via transciliary supraorbital keyhole craniotomy depend on residual tumor volume. The extent to which pathology and size of tumor influence the resection rate using this approach is unknown. Sixty-two patients underwent a total of 64 operations using the supraorbital keyhole approach in this retrospective study. Meningioma was the most common tumor, followed by pituitary adenoma and craniopharyngioma. Age, sex, tumor volume, operative duration, blood loss, and complication rates were evaluated. Pre- and postoperative residual tumor volumes were measured using OsiriX software (medical image viewer system) based on magnetic resonance imaging. A 15-mL cut value divided the subjects into large versus small meningioma groups. The average resection rate for meningiomas was 95.2% compared with 83.9% for craniopharyngiomas and 53.2% for pituitary adenomas. The major complication rate (primarily blindness and hemiplegia) was 4.48% in all tumors. No operative-related deaths occurred. There were no surgical revisions to traditional large craniotomies. No significant differences in age, sex, postoperative volumes, resection rates, or recurrence rates were noted between small and large meningioma groups. However, longer operative times and hospital stays, and greater blood loss occurred in the large meningioma group. Transciliary keyhole craniotomy is a safe and effective approach for anterior skull base tumors, especially meningiomas. Excellent resection results were achieved even in cases of large meningiomas. Although longer operative times, longer hospital stays, and greater blood loss occurred in larger compared with smaller meningioma cases, recurrence rates were similar.

SURG-13. THIRD HARMONIC GENERATION (THG) IMAGING: A NOVEL TOOL FOR INTRA-OPERATIVE HISTOLOGIC ANALYSIS OF FRESH HUMAN GLIOMA TISSUE

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BACKGROUND: Label-free optical techniques such as optical coherence tomography and Raman techniques are reported to hold promise for improved surgical management of central nervous system (CNS) tumors because of their high resolution and intra-operative applicability in fresh tissue samples. We have recently reported on third harmonic generation (THG) imaging as an alternative, label-free tool for fast imaging of histologic characteristics in fresh, unprocessed human tissues. AIM: To determine the potential of THG microscopy for assessment of tumor load in unprocessed tissue samples of patients with a diffuse glioma. MATERIALS & METHODS: Freshly obtained CNS tissue samples (n = 55) of 23 patients with diffuse glioma were analyzed using THG microscopy and subsequently processed for routine pathological analysis. Later on, the histology as seen in H&E-stained sections of these samples was correlated with the THG images. Furthermore, using automated image analysis, an algorithm was developed for classification of the THG images as tumor versus non-tumor in order to further facilitate exploitation of THG microscopy as a real-time, intra-operative diagnostic support tool. RESULTS: In all samples, THG microscopy revealed histology-grade characteristics with information on a spectrum of cellular and extracellular components. In most tumor-containing samples, THG imaging readily allowed for visualization of (a gradient of invasive) tumor cells in the CNS parenchyma. Interpretation of the THG images by automated, quantitative image analysis, with particular emphasis on cell density and composition of the neuropil, provided an observer-independent interpretation of the same images. While in depth comparative analysis is still ongoing, preliminary results of comparison of (qualitative and quantitative) information in THG and H&E images are very promising. CONCLUSIONS: THG microscopy enables intra-operative visualization of histopathological characteristics in unprocessed human brain tissue. Qualitative and quantitative analysis of THG images of ex-vivo (and ultimately in-situ) brain tissue has great potential for improving CNS tumor surgery.

SURG-14. ANALYSIS OF TREATMENT RESULTS FOR RECURRENT GLIOBLASTOMA INCLUDING IMMUNE STATUS ALTERATION

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This study was designed to analyze our treatment results for recurrent GBM (rGBM) and to investigate the change of molecular expression, including C3, programmed death-1 (PD-1), and programmed death-ligand 1 (PD-L1) on paired primary and recurrent tumor specimens of GBMs and to evaluate the influence of their changes for patients survival. From 2004 to 2015, 170 patients with rGBM were included. Forty-three patients (25.3%) were selected for 2nd operation upon recurrent disease (re-operated group) and 127 patients did not undergo 2nd operation (non-operated group). We also evaluated immunohistochemical expression of immunologic markers of 43 paired surgical specimens from the 1st and 2nd operation. Median overall survival after recurrence (rOS) of re-operated group showed significant longer than that of non-operated group (median: 9.1 months vs 5.6 months, P=0.024), The re-operated group showed relative younger age, better Karnofsky performance scale (KPS), and lower rate of leptomeningeal seeding, and eloquent area involvement. In re-operated group, higher KPS and extent of resection was significantly associated with longer rOS. Among 43 paired surgical specimens from the 1st and 2nd operation, positive expression of PD-L1 was 17 patients (39.5%) after 1st operations and 6 patients (13.9%) after 2nd operations. Changes of PD-L1 expression rate after recurrence were as follows; increased group (n=5, 11.6%), decreased group (n=13, 30.2%), and no change group (n=25, 58.1%). The PD-L1 expression and changes of PD-L1 expression did not affect the survival after recurrence. C3 and PD-1 tumor infiltrating mononuclear cells were not detected in almost all initial and secondary specimens. In some selected patients with a recurred GBM, re-operation could be good treatment option. The PD-L1 expression from the 1st and 2nd operation and changes of PD-L1 expression after recurrence did not influence on survival for GBM patients. However, it needs further investigations to reveal the role of immunity in GBM.

SURG-15. UPFRONT MRI-GUIDED STEREOTACTIC LASER-ABLATION IN NEWLY DIAGNOSED GLIOBLASTOMA: A MULTICENTER REVIEW OF SURVIVAL OUTCOMES COMPARED TO A MATCHED COHORT OF BIOPSY-ONLY PATIENTS

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BACKGROUND: Laser ablation (LA) is used as an upfront treatment in patients with deep seated newly diagnosed Glioblastoma (nGBM). We evaluated the outcomes of LA in patients with nGBM and compared them with a matched biopsy-only cohort. METHODS: 24 nGBM patients underwent upfront LA at Cleveland Clinic, Washington University in St. Louis and Yale University (6/2011–12/2014) followed by chemo/radiotherapy (CRT). Also, 24 out of 171 nGBM patients with biopsy from Duke and Yale Universities who followed by CRT were matched with our LA cohort based on age (<70 vs 70), gender, tumor location (deep vs lobar) and volume (<11 cc vs 11 cc). Progression-free survival (PFS), overall survival (OS), disease-specific PFS and OS ((DS-PFS, DS-OS) were our endpoints. Three prognostic groups were identified based on extent of tumor ablation by thermal-damage-threshold (TDT)-lines. RESULTS: The median tumor volume in LA (n=24) and biopsy

only (n=24) groups was 9.3 cm3 and 8.2 cm3 respectively. Overall, median estimate of OS and PFS in LA cohort was 14.4 and 4.3 months compared to 15.8 months and 5.9 months for biopsy only cohort. On multivariate analysis, favorable TDT-line prognostic groups were associated with lower incidence of disease specific death (p=0.03) and progression (p=0.05) compared to other groups including biopsy only cohort. Only age (<70 years, p=0.02) and tumor volume (<11 cc, p=0.03) were favorable prognostic factors for OS. CONCLUSIONS: The maximum tumor coverage by laser ablation followed by radiation/chemotherapy is an effective treatment modality in patients with newly diagnosed GBM who are not a candidate for standard craniotomy, compared to biopsy only cohort. The TDT-line prognostic groups were independent predictor of disease specific death and progression after laser ablation.

SURG-16. MRI NEURONAVIGATION-GUIDED RESECTION FOR RECURRENCE-INITIATING GBM CLONES.

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Diffuse infiltration is a hallmark of Glioblastoma (GBM) and surgical resection of the entire tumor cells is practically impossible. Residual tumor cells at surgery retain a subpopulation that initiates life-threatening tumor recurrence, termed recurrence-initiating cells (RICs). From the standpoint of spatial distribution, given that the tumor cells in the gadolinium-enhancing lesions (tumor core) can largely be removed at surgery, RICs are supposedly located at the non-enhancing edge lesions with T2-FLAIR changes. Although intratumoral molecular and phenotypic heterogeneity is representative of malignant tumors such as GBM, the edge-located RICs have not been well-defined due to the difficulty in safe resection. As a neuro-oncology surgeon, I have developed a means to molecularly and phenotypically characterize the edge-located RICs by combining the Stealth MRI-guided localized tumor resection at surgery, followed by clonal characterization of tumor cells in my laboratory. In this presentation, I will summarize the strength and limitations of this established approach.

SURG-17. COMBINED AWAKE CRANIOTOMY AND TRANSCORTICAL MEP FOR RESECTION OF MOTOR AREA GLIOMAS

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BACKGROUND: Resection of primary motor area gliomas have risk of severe motor deficits and it is considered difficult to massive removal. We have actively resected gliomas in this area with combined awake craniotomy and transcortical motor evoked potentials (MEP) since 2005. We presented the removal method and surgical results for tumor in this area, and examined factors related to postoperative motor deficits. METHODS: The present study included 30 consecutive patients (nine men and nine women; mean age 40 years) with a primary motor area glioma from 2005 to 2017. All tumors were removed with confirming spontaneous movement during awake craniotomy and monitoring transcortical MEP. Postoperative motor deficits were evaluated in four categories: Stable, Declined (Mild, Moderate, Severe). We defined Moderate and Severe as deficits that interfere with daily life. RESULTS: In 28 of 30 cases, an effective waveform was not obtained with transcortical MEP. The mean extent of resection was 93%. Motor deficits at 6 months after surgery were Stable 20, Mild 7, Moderate 2 cases, and Severe one case. Moderate and Severe deficits were confirmed in 3/30 cases (10%). Motor deficits at 6 months after surgery was significantly correlated with declined intraoperative spontaneous movement, decreased transcortical MEP more than 50%, and presence of ischemic lesion postoperative MRI. The patients without declined spontaneous movement with/without decreased transcortical MEP showed no decline of motor function 6 months after surgery (6 patients). While, those with declined spontaneous movement without decreased transcortical MEP showed 20% (2 of 10 patients) of declined cases, and those with declined spontaneous movement with decreased transcortical MEP more than 50%, 67% (8 of 12 patients) of declined cases. CONCLUSIONS: Monitoring of spontaneous movement during awake craniotomy and neurophysiological monitoring by transcortical MEP have complementary relationship, are useful for removal of primary motor area gliomas, and contribute aggressive removal.

SURG-18. COMPARISON OF SURVIVAL OUTCOMES BETWEEN PARTIAL RESECTION AND BIOPSY FOR PRIMARY GLIOBLASTOMA: A PROPENSITY SCORE-MATCHED STUDY

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OBJECTIVES: Gross total resection (GTR) for glioblastoma (GBM) is associated with a better prognosis. However, GTR is not always feasible and the threshold of the extent of resection for better prognosis is controversial. Therefore, we compared the survival and clinical outcomes of GBM between partial resection (PR) and biopsy. Methods Thirty-two and 78 patients who underwent PR and biopsy, respectively, were enrolled this study to identify differences in clinical outcomes. There were no differences in patient demographics between the PR and biopsy groups except for tumor location and mean tumor volume (p=0.02 and <0.01, respectively). Propensity score matching (PSM) between the PR and biopsy groups was performed, in which 20 patients each were matched in the PR and biopsy groups. Results The overall survival (OS) and progression-free survival (PFS) did not differ significantly between the PR and biopsy groups (p=0.84 and 0.48, respectively). After PSM, the difference in OS and PFS difference between the groups was not statistically significant (p=0.51 and 0.75, respectively). The hazard ratios for OS and PFS of PR to biopsy were 0.98 and 0.73, respectively; however, the difference was not statistically significant (p=0.96 and 0.39, respectively). The surgical complication rate was higher in the PR group (14 of 32, 43.7%) than that in the biopsy group (9 of 78, 11.5%) (p<0.01). Conclusion PR failed to improve survival compared to biopsy in patients with GBM. Moreover, the surgical complication rate in the PR group was higher than that in the biopsy group.

SURG-19. IMPACT OF INTRAOPERATIVE MAGNETIC RESONANCE IMAGING ON THE EXTENT OF RESECTION AND FUNCTIONAL OUTCOME IN AWAKE SURGERY FOR ELOQUENT GLIOMAS – A SINGLE CENTER RETROSPECTIVE STUDY

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Maximizing the extent of resection (EOR) while preserving functional integrity is a mainstay of glioma surgery. Intraoperative MRI (iMRI) helps to augment the EOR. However, in eloquently located gliomas the significance of iMRI is controversial since the EOR is limited by functional rather than image-based boundaries. Thus, we sought to determine the impact of iMRI in our institutional series of awake glioma resections within or adjacent to eloquent (language, motor, sensory) areas since the implementation of a 1.5 Tesla iMRI in 2009. Tumor- and procedure-related data and functional outcome were assessed through medical charts review. The EOR was determined volumetrically on pre-, intra- and postoperative T1 contrast-enhanced (CE) and FLAIR MR images. 86 of 104 awake surgeries (83%) were performed under iMRI-guidance with concurrent language (n=72) and/or motor (n=50) mapping. iMRI was done when functional boundaries were reached (n=26), for resection control (n=53) or for other reasons (n=7). Additional resection after iMRI (AR) was performed in 63 cases (73%); otherwise resection was terminated because the targeted EOR or functional boundaries were reached. New or deteriorated neurological deficits occurred in 20 patients prior and 15 patients post iMRI; however, all but 3 resolved within 6 months. Median EOR significantly increased after AR from 92.6% to 98.4% (5.8%; p<0.0001) in CE tumors and from 64.5% to 85.8% (21.3%; p<0.0001) in non-enhancing tumors. Remarkably, the reason to perform iMRI (resection control or functional limitations), did not affect the frequency of AR, deficits acquired post iMRI or the increase in EOR after AR. In conclusion, iMRI is a valuable adjunct to maximize the EOR in awake glioma resections without increasing the risk for functional impairment, particularly in non-enhancing tumors. Importantly, iMRI contributes to a maximized EOR even in cases where the resection had to be stopped because functional boundaries were reached.

SURG-20. LASER-INTERSTITIAL THERMAL THERAPY VERSUS CRANIOTOMY FOR TREATMENT OF RADIATION NECROSIS OR RECURRENT TUMOR IN BRAIN METASTASES FAILING RADIOSURGERY

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INTRODUCTION: Laser-interstitial thermal therapy (LITT) and craniotomy are both options for treatment of radiation necrosis (RN) and re-growing tumor (RT) occurring after radiosurgery for brain metastases. No studies to-date have compared the two options. METHODS: We performed a single institution, single surgeon retrospective study of previously irradiated brain metastases treated by LITT or craniotomy between

February 2007 and September 2016. Data collected included demographics, steroid dosing, neurological outcomes, local progression-free survival (PFS), and overall survival from surgery date. Categorical variables and outcomes were analyzed with Fishers exact and log-rank tests, respectively. RESULTS: Of 75 patients, 42 had RT (56%) and 33 (44%) purely RN. Of patients with RT, 26 underwent craniotomy and 16 LITT. For RN, 15 had craniotomy and 18 LITT. No significant difference was found between LITT and craniotomy in ability to taper off steroids (p=0.53), including ability to initiate or resume immunotherapy post-operatively in melanoma patients (craniotomy: 10/16, 62.5% versus LITT: 8/11, 72.7%; p=0.69). PFS and OS were similar for LITT versus craniotomy, respectively: %PFS at 1-year = 72.2% versus 61.1%, %PFS at 2-years = 60.0% versus 61.1%, p=0.72; %OS at 1-year = 69.0% versus 69.3%, %OS at 2-years = 56.6% versus 49.5%, p=0.90. Craniotomy resulted in higher rates of pre-operative deficit improvement than LITT (p<0.01). On subgroup analysis, patients undergoing craniotomy for RN trended towards improved PFS compared with those undergoing LITT for RN or those with tumor (regardless of local treatment option). CONCLUSION: LITT appears to be as efficacious as craniotomy in achieving local control of recurrent irradiated brain metastases and facilitating steroid taper, regardless of pathology, including post-operative initiation or resumption of immunotherapy in melanoma patients. Craniotomy, however, is more advantageous in relieving those with pre-operative symptoms and may provide a survival advantage in those with radiation necrosis.

SURG-21. SUPRACEREBELLAR TRANSTENTORIAL APPROACH FOR OCCIPITAL MENINGIOMA TO MAXIMIZE VISUAL PRESERVATION: TECHNICAL NOTE

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BACKGROUND: Surgery for resection of tentorial meningiomas that compress the primary visual cortex has a significant risk of worsening vision. This concern is especially acute in patients with a preexisting visual deficit. Approaches that involve mechanical retraction of the occipital lobe further threaten visual function. The supracerebellar transtentorial (SCTT) approach, which does not carry a risk of retraction injury, should be considered for occipital tentorial meningioma to maximize functional visual outcomes. CLINICAL PRESENTATION: A 54-year-old woman underwent two resections and radiation therapy for a right occipital oligodendroglioma as a teenager. She was left with a complete left homonymous hemianopsia. The patient now presented with progressive vision loss in her remaining right visual field. Imaging revealed a left occipital superiorly projecting tentorial meningioma. Given that preserving remaining visual function was of critical importance in this case, the SCTT approach with the patient in the prone position was chosen for resection. Traditionally favored suboccipital lateral and occipital interhemispheric supratentorial approaches risked retraction injury to visual cortex and further visual loss. A transcortical or transfalcine approach, also considered given access via the large right occipital resection cavity, would similarly require manipulation of the remaining medial rim of left occipital lobe. A Simpson grade 1 resection was achieved without disrupting the occipital lobe pia or requiring cerebellar retraction while minimizing intraoperative risk of air embolism. A diagnosis of a WHO grade II meningioma (presumably radiation induced) was made. The patients vision returned to pre-morbid baseline one week after surgery. CONCLUSION: The SCTT approach should be considered for the surgical management of patients with occipital tentorial meningiomas when visual preservation is at risk. This approach avoids transgression of visual cortex, and minimizes the risk of venous infarction or contusions from retraction injury.

SURG-22. SUPRATOTAL RESECTION IN GLIOMA: A SYSTEMATIC REVIEW

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PURPOSE: To evaluate the published evidence addressing supratotal resection in glioma, where supratotal is defined as resection beyond all MRI abnormalities present on T1 enhanced and FLAIR modalities. METHODS: EMBASE, MEDLINE, Scopus, and Web of Science were queried using search terms designed to identify published works on supratotal resection. Records that were case studies, reviews or editorials, non-English, abstract-only, brain metastases, or only descriptive, were excluded. All others were included. RESULTS: 309 unique references yielded 41 studies for full-text review, with 7 included in the final analysis. Five originated from research in France, one from Germany, and one from Italy. All five French studies focused on low-grade glioma, whereas the remaining two focused on glioblastoma. A total of 88 patients had undergone supratotal resection in a combined cohort of 492 patients (214 males and 278 females, age 18 to 82 years). Fifty-one supratotal resections were conducted on high-grade gliomas, and 37 on low-grade gliomas. Surgical resection technologies included intraoperative MRI, cortical and subcortical functional testing, and 5-aminolevulinic acid. Studies were mostly of Oxford Center for Evidence-Based Medicine Level 4 quality. Karnofsky Performance Status, overall survival, progression-free survival, neurological deficits post-operatively, and anaplastic transformation were the main measured outcomes. No randomized controlled trials were identified. Preliminary low quality support was found for supratotal resection in increasing overall survival and progression-free survival for both low-grade and high-grade glioma. CONCLUSION: The literature suggests insufficient evidence for *carte blanche* application of supratotal resection, particularly in lower grade gliomas where neurological deficits can result in long-term disability. Current evidence consists of data from only a few centers without independent validation. There is a definite need for further research with larger patient populations, clearly defined metrics and comparisons to evaluate improvements by, and the involvement of multi-national/multi-center research groups.

SURG-23. CLINICAL RESULTS AFTER SURGICAL RESECTION OF PARASAGITTAL MENINGIOMAS IN PATIENTS USING A ND-YAG LASER IN THE DISTAL POSTOPERATIVE PERIOD

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Nowadays, the problem of surgical treatment of cerebral meningiomas of complex localization in the whole world remains relevant. First, it is associated with a high risk of relapse and continued growth. Secondly, subsequent re-surgeries often lead to the development of a persistent neurological deficit and disability of patients, thereby significantly reducing the quality of life. The use of a neodymium laser during the removal of the tumor node and the processing of the meningioma matrix leads to a statistically significant reduction in postoperative complications in the early postoperative period, and the number of patients with relapses and continued growth is statistically significantly reduced, which affects the quality of life in the remote postoperative period. In this article, we analyzed the dynamics of the neurological deficit, the frequency of relapse and continued growth, as well as the analysis of the dynamics of the functional state of the brain and cerebral hemodynamics in the distal postoperative period. This analysis was carried out in two statistically homogeneous groups of patients operated on for parasagittal meningiomas. In the first group, Nd-YAG laser radiation was used in parallel (study group). In the second group (control group) operated by conventional microsurgical techniques remove cerebral meningiomas. The distant period in the study group received the significant reduction of neurological deficiency, the number of recurrences, as well as marked improvement in functional status and cerebral hemodynamics and hence the highest quality life of the patients in this group.

SURG-25. INTRAMEDULLARY SPINAL CORD TUMOURS – A SINGLE UNITED KINGDOM CENTRE TEN-YEAR ANALYSIS

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BACKGROUND: Intramedullary spinal cord tumours are relatively rare tumours of the central nervous system. Surgical outcomes are affected by many variables, including pre-operative neurological function, tumour histology and extent of resection. Emphasis remains on surgical treatment due to the limited adjunctive therapeutic options and poor drug penetration. OBJECTIVE: To identify clinically relevant predictors of progression free survival by retrospectively analysing the anatomical location, pre- and post-operative function and histology in intramedullary spinal cord tumours from a single United Kingdown neurosurgical centre over 10 years. METHOD: 49 patients were identified from a surgical database. Variables collected included pre-and post-operative Frankel Grade and Modified McCormick Scale assessments, tumour histology, extent of resection and length of follow up. Chi-Squared, Kaplan-Mier Survival and Mann-Whitney U-Tests were completed. RESULTS: Ependymoma, Haemangioblastoma and Pilocytic Astrocytoma were the commonest tumour histologies. In total 21 different histological tumours were identified in the series. There was a statistically significant relationship between identification of the tumour plane and extent of resection (p<0.01), along with the extent of resection and recurrence (p<0.01). Compared to the other histological subtypes, ependymoma's demonstrated a significantly greater extent of resection (p=0.02). There was a significant relationship between the grade of tumour and progression free survival (p<0.01). We did not find a significant relationship between pre- and post-operative neurological function and survival. CONCLUSION: Tumour plane and the extent of tumour resection are significant determinants of progression free survival. Ependymoma, whilst being the commonest histology in our series were also the most resectable. Whilst complete resection reduces the rate of recurrence, tumour grade is the most important predictor of outcome.

TUMOR MICROENVIRONMENT

TMIC-01. LOX DEPENDENT MACROPHAGE RECRUITMENT IN GBM

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Glioblastoma (GBM) is the most lethal form of brain cancer in adults with no effective therapeutics. Genomic profiling has stratified GBM into various subgroups, which are driven by specific genetic alternations of core signaling pathways, including RTK/RAS/PI-3K, P53 and RB pathways. The main reason for this failure is tumor-cell genetic heterogeneity, which induces aberrant activation of multiple signaling pathways. Stromal/immune cells in the tumor microenvironment (TME) are genetically stable, which not only play a pivotal role in GBM progression by affecting multiple cancer hallmarks, but can also be educated by cancer cells. However, whether and how the behavior and function of specific stromal/immune cells in the TME are regulated by genetic alternations in GBM remain relatively undefined. Here, we show that PTEN deficiency in GBM specifically triggers immune response by promoting macrophage recruitment, while without affecting macroglia and other immune cells. Using unbiased transcriptome profiling, we identified that lysyl oxidase (LOX) is preferentially secreted by PTEN-deficient cancer cells, and is a potent macrophage chemoattractant. Transcriptome profiling following Gene Set Enrichment Analysis and functional validation demonstrated that activation of SRC and AKT signaling pathways drives LOX upregulation in PTEN-deficient cancer cells, thus promoting macrophage recruitment. Following internalization into macrophages mediated by integrin b1, LOX promotes macrophage migration via a newly identified signaling pathway through phosphoprotein profiling. Genetic and pharmacological inhibition of LOX in PTEN-deficient cancer cells does not affect tumor cell proliferation in vitro, but markedly inhibits macrophage density and tumor growth in vivo. Clinically, LOX expression positively correlates with integrin b1, as well as macrophage signature and poor prognosis in GBM patients. LOX-integrin b1 axis is enriched in GBM patients with higher macrophage density, and that these patients show lower survival. Together, our study highlights the possibility of improving GBM treatment by targeting PTEN-LOX axis-mediated macrophage recruitment.

TMIC-02. INTERACTION OF LIGAND CONJUGATED QUANTUM DOTS WITH THE GLIOMA STEM CELL SECRETED EXOSOMES AND SUBSEQUENT UPTAKE BY THE GLIOMA STEM CELLS OF VARIOUS SUBTYPES

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Glioblastoma tumors are known to secrete an enormous number of exosomes with various molecular signatures. Some recent studies also demonstrate that the exosomes secreted by the glioma initiating cells or glioma stem cells carry differentially expressed mRNA, miRNA and protein that would impart radiation and chemo-resistance to the adjacent non-stem cancer cells. It is important to investigate the exosome secretion profile by the cancer stem cells and their re-uptake profile by the glioma cells belonging to various subtypes in the vicinity of tumor. In our earlier study we identified that exosomes from glioma stem cells express a tumor associated IL13Rα2 that could bind with interleukin 13 conjugated quantum dots (QD), which can be subsequently profiled by various physico-chemical methods. In our recent investigation we observed that a mutated form of IL-13, TQM13, can be conjugated with QD and can complex with exosomes. These QD-bound exosome can subsequently be monitored by fluorescent microscopy utilizing the intrinsic fluorescence property of the quantum dots. In our present investigation,TQM13-QD was complexed with exosomes that originated from glioma stem cells from mesenchymal (T387) and proneural (T3691) subtypes. Various glioma cell lines such as U87, T3691 and T387, and Jurkat cells, an immortalized T-lymphocyte cells, were then treated with the TQM13-QD:exosome complex. Our investigation revealed that cells had a higher uptake of the TQM13-QD upon complexation with exosomes when compared to quantum dots without exosome complexation. In vivo experiments were subsequently performed by injecting T3691 exosomes complexed with TQM13-QD in U87-Luc cells which were implanted as an orthotopic glioma tumor mouse model. Intravital imaging spectroscopy (IVIS) images confirmed the localization of TQM13-QD: exosome complex in the tumor region as early as 60 minutes post injection. Current studies are investigating the mechanism of uptake of TQM13-QD complexed with fluorescently labeled exosomes and quantifying their uptake.

TMIC-03. DISSECTING GLIOMA:IMMUNE CELL INTERACTIONS AT THE SINGLE CELL LEVEL

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High grade gliomas such as glioblastomas (GBMs) remain virtually incurable diseases. Recent success with immunotherapies for other solid tumors has demonstrated the power of harnessing one's own immune system to combat malignant cancers. However, on-going clinical trials using checkpoint inhibitors and other approaches show disappointing results in glioma patients. A major known hurdle for many immune therapies is the highly immune-suppressive glioma microenvironment. There are large numbers of myeloid derived suppressive cells (MDSCs) and tumor associated macrophages (TAMs) that promote glioma growth directly and indirectly by block recruitment and infiltration of anti-tumorigenic immune cells (such as T cells). Another potential challenge is the heterogeneity of glioma:immune interactions that generate nuanced but functionally significant differences in different regions of the same patient tumor. To better understand the microenvironmental heterogeneity and glioma:immune cell interactions in different microenvironments, we analyzed cell:cell communication among GSCs, non-stem glioma cells, MDSCs and TAMs in human GBM tissues at the single cell level. We sampled multiple regions from individual GBM tumors during surgical resection to address spatial heterogeneity in immune infiltration and glioma cell genotypes and phenotypes. We performed single cell RNA-sequencing (scRNA-seq) and exome-sequencing as well as immune phenotyping by flow analyses from each fragment separately. Then, using a novel systems biology tool we recently developed, Cell-to-cell Communication Explorer (C2CCE), we systematically analyzed cell-to-cell communication among glioma cells and immune cells at the single cell level. We observed striking spatial heterogeneity of immune infiltration in some patients. We also observed specific cell:cell communication hierarchy among glioma cells, TAMs, and MDSCs. We are currently working on identifying different communication channels between glioma stem cells and non-stem cells with different immune cell types.

TMIC-04. NONFUNCTIONAL PITUITARY ADENOMAS DEMONSTRATE TWO SUBTYPES BASED ON MACROPHAGE POLARIZATION STATUS

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INTRODUCTION: Nonfunctional pituitary adenomas (NFPAs), while typically benign, display variable invasiveness, and can present with devastating symptoms of intercranial mass effect. Tumor-associated macrophages (TAMs) have long been accepted as active participants in tumor microenvironments, particularly their M1 and M2 subtypes, correlating with anti- and pro-tumoral effects respectively. We investigated TAMs in NFPA biology and clinical behavior. METHODS: Patient NFPAs were FACS sorted for total macrophages and M1 and M2 subtypes. Functional assays utilized monocyte cell line THP1. RESULTS: Macrophage isolation from fourteen NFPAs revealed TAMs average 8.5% of dissociated cells (range=0.5%-29.1%), displaying bimodal distribution with most cases clustering below 8% CD11b fraction. Two cases exhibited 18–29% CD11b fractions and were more expansile (size>3.5 cm or MIB1>3%). M2/M1 gene expression ratios assessed by qPCR were above one in NFPAs with cavernous sinus invasion, including the TAM-rich expansile tumors, while M2/M1 ratios were below one in NFPAs without cavernous sinus invasion. Increasing TAM percentage correlated with decreased M2/M1 ratio determined by qPCR of subtype-specific TAM markers. THP1 cells were polarized and used to created media conditioned with M1 and M2 TAMs. Primary cultured NFPA patient cell lines showed that M2 conditioned media induced more invasion ($P < 0.01$), migration ($P < 0.05$) and proliferation ($P < 10^{-3}$). qPCR screening of patient cell lines identified S100A9 and EZH2 as mediators of M2-induced invasion and proliferation, respectively. CONCLUSION: Our work identified two subtypes of NFPAs based on TAM status: (1) Larger, more proliferative NFPAs exhibited increased TAMs and decreased M2/M1 ratio but sufficient M2-induced EZH2 can drive proliferative expansion with sheer TAM numbers; and (2) NFPAs invading the cavernous sinuses exhibited lower TAMs and higher M2/M1 ratios, with M2-induced S100A9 driving invasion. These results suggest that recruitment of TAMs to the pituitary microenvironment influences tumor development and M2 TAMs promote a more pro-tumoral state.

TMIC-05. ABSCOPAL IMMUNE RESPONSE IN GLIOBLASTOMA ELICITED BY MIR124-ATTENUATED ONCOLYTIC HERPES SIMPLEX VIRUS 1 ARMED WITH UL16 BINDING PROTEIN 3

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Glioblastoma is a deadly disease with median survival ranging below one year. T-cell-based immunotherapy approaches failed to prolong survival of glioblastoma patients in two phase III clinical trials, thus reflecting the immunologically inert features of glioblastoma. Pre-clinical efficacy of oncolytic virus therapy in combination with immune checkpoint inhibition was demonstrated in syngeneic glioblastoma models. However, given the extensive spreading of glioblastoma cells throughout the brain, we hypothesized that efficacious immunotherapy approaches in patients will need to elicit abscopal responses, i.e. the secondary targeting of tumor cells at sites distant from treated lesions. We addressed this issue in an immune competent *RCAS/tv-a*-based genetic glioblastoma model that recapitulates the genotype, gene expression, histology and immune phenotype of human glioblastomas. Spatial distance of virus-treated and untreated glioblastoma lesions was modeled by generating two tumors in both hemispheres of *N/tv-a;Ink4a/Arf*$^{-/-}$*;PTEN*$^{fl/fl}$*;Luc*$^{LSL/LSL}$mice. One tumor was generated by transduction with *RCAS-Pdgfb* and *RCAS-shPTEN*. A second, contralateral tumor was generated with *RCAS-Pdgfb* and *RCAS-Cre*, thus enabling additional monitoring of tumor growth through luciferase activity. We infected luciferase-negative tumor lesions with a miR-124-attenuated oncolytic herpes simplex virus 1 (oHSV) armed with the NKG2D ligand ULBP3. oHSV-ULBP3 infection slowed the growth of distant, non-infected glioblastoma lesions and prolonged survival. oHSV-ULBP3 led to influx and activation of T-cells in both, treated and untreated glioblastoma lesions. Massive influx of monocytes yielded an increase of mostly PD1+ macrophages in both tumors. Neutrophils were also increased ubiquitously, whereas NK-cells were suppressed. Treatment with an anti-PD1 antibody alone had no effect, but combination with oHSV-ULBP3 counteracted macrophage and neutrophil influx and enhanced abscopal anti-tumor responses. We conclude that oHSV-ULBP3 in combination with anti-PD-1 is a feasible approach to induce abscopal T-cell-mediated immune responses in glioblastoma. Unraveling the role of myeloid components of the tumor microenvironment may be critical to overcome resistance to such treatment.

TMIC-06. DEFICIENCY OF miRNA-146a-5p CONTRIBUTES TO TRANSFORMATION OF GLIOMA-DERIVED MESENCHYMAL STROMAL/STEM CELLS VIA TARGETING HNRNPD

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Although malignant transformation of tumor stroma cells has been reported previously, its relevant mechanisms still kept largely unknown. In this study, we established three intracerebral xenograft tumor models byde novo transplanting glioma initiating cells and glioblastoma surgical specimens into cerebral cortex of the enhanced green fluorescent protein (EGFP) transgenic nude mice and chimeric nude mice. With dual color tracing techniques, which based on transfection of tumor cells with red fluorescent protein(RFP) and host cells bearing EGFP, three EGFP-expressing host bone marrow derived mesenchymal stromal cells (MSCs) were sorted from different intracerebral xenograft models, showing characteristics of immortality and malignant growth pattern. High-throughput RNA-sequencing of these cells results showed up-regulation of 19 miRNAs, and down-regulation of 24 miRNAs, respectively. MiRNA-146a-5p was one of the downregulated miRNAs, and overexpression of it can reverse malignant phenotype of transformed MSCs. Double-luciferase assay showed that miRNA-146a-5p directly acted on oncogene HNRNPD which was overexpressed in cancerous MSCs. SiRNA targeting HNRNPD could efficiently inhibit the proliferation of transformed cells and arrest the cell cycle at G2/M phase, and increase the apoptosis of cells. In summary, deficiency of miRNA-146a-5p contributes to the malignant transformation of host bone marrow derived mesenchymal stromal cells in the intracerebral xenograft tumors by overexpressing the tumorigenesis gene, HNRNPD.

TMIC-07. HYPOXIC MICROENVIRONMENT CONFERS SPECIFIC ALTERATIONS IN DNA METHYLATION PROFILES IN GLIOBLASTOMA

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High cellularity and poorly organized tumour vasculature in high-grade gliomas leads to insufficient blood supply, hypoxic areas, and ultimately to the formation of necrosis. Thus, hypoxia is a hallmark of malignant glioma microenvironment and it is associated with aggressive tumor behavior such as growth, progression, and resistance to chemo-radiation. Current pathologic markers are insufficient to identify patients that may benefit from specific treatments. We therefore, hypothesized that underlying epigenetic alterations confer therapeutic resistance under hypoxic conditions. Twenty five GBM patients were consented and treated with pimonidazole (PIMO) 16–18 hours prior to surgery. Tumor sections were subjected to immunohistochemical analysis using antibodies against PIMO and other hypoxia markers such HIF1a and CAIX. Samples were subjected to laser capture microdissection followed by DNA isolation and DNA methylation profiling using the Illumina Human Methylation EPIC Array. Data was analyzed using *minfi* and *conumee* packages in Bioconductor, together with appropriate biostatistics tools. PIMO score was determined to range from 10–60% and positively correlated with other hypoxia markers such as CA IX and HIF1a (p4,000) were hypomethylated. Gene set enrichment analysis (GSEA) indicated that the majority of these CpGs are associated with genes involved in signalling cascades and oncogenic processes, including WNT and NOTCH. These were compared to DNA methylation profiles of glioma stem cells exposed to transient hypoxia and extensive overlap was found in proportion of hypomethylated CpG sites and cellular processes that were altered. These findings were correlated with complementary RNA expression data from RNA sequencing to establish the biological relevance of changes in DNA methylation profiles under hypoxia in GBM.

TMIC-08. CHD7 IS SUPPRESSED IN THE PERINECROTIC/ISCHEMIC MICROENVIRONMENT AND IS A NOVEL REGULATOR OF ANGIOGENESIS

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A pathologic hallmark of glioblastoma (GBM), the most common and deadly primary brain tumor in adults, is pseudopalisading necrotic regions in which ischemic conditions are found. Ischemic microenvironments characterized by low oxygen, restricted glucose, and acidic pH due to poor blood supply occurs in both solid tumors and non-neoplastic tissue injury. Modeling these physiologically relevant ischemic conditions within glioblastoma in vitro, we identified chromodomain helicase DNA binding protein 7 (CHD7) as a novel ischemia-regulated gene. CHD7 is an epigenetic reader that controls neural stem cell maintenance and is mutated in CHARGE syndrome, a developmental disorder associated with cranial nerve abnormalities. CHD7 mRNA expression was consistently suppressed in multiple patient-derived xenograft brain tumor lines in response to ischemic conditions, and lower expression of CHD7 correlates with poor GBM patient survival. These microenvironment-mediated decreases in CHD7 protein and mRNA levels observed in glioblastoma were also mirrored in neural progenitor cells in vitro, and CHD7 levels were reduced in the perinecrotic niche of GBM patient and xenograft tissue sections. Genetic targeting of CHD7 increased angiogenesis in vitro in association with differential regulation of angiogenesis associated genes as determined in RNA sequencing analysis. Although targeting CHD7 in orthotopically implanted xenograft brain tumor models did not affect survival outcomes in mice, we observed marked differences in blood vessel architecture and organization, suggesting a major role for CHD7 in regulating vessel formation within these tumors. Together, our data provide insight into the molecular responses to ischemia that regulate angiogenesis.

TMIC-09. EXCITATORY SYNAPSES BETWEEN PRESYNAPTIC NEURONS AND POSTSYNAPTIC GLIOMA CELLS PROMOTE GLIOMA PROGRESSION

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Neuronal activity robustly regulates high-grade glioma growth. Here, we report that neuron-glioma interactions include bona fide synaptic communication. Glioma cell expression of synapse-related genes was confirmed at the single cell level in primary adult and pediatric glioma samples. Structural synapses between presynaptic neurons and post-synaptic glioma cells were identified by confocal and electron microscopy. Voltage clamp recordings from patient-derived glioma cells xenografted to the CA1 region of the hippocampus demonstrates AMPAR-mediated excitatory neurotransmission between presynaptic neurons and post-synaptic glioma cells with local electrode stimulation of inputs to the CA1 region. Millisecond timescale excitatory post-synaptic currents (EPSCs) were observed in approximately 10–20% of glioma cells xenografted to the hippocampus. In some patient-

derived glioma xenograft models, a larger subpopulation of glioma cells exhibit a second electrophysiological profile with longer, ~1 sec depolarization in response to neuronal activity. These longer timescale depolarizations induced by neuronal input activity are blocked by gap junction inhibitors, supporting the concept that neuron to glioma excitatory neurotransmission can be communicated through gap junction-coupling in a network of glioma cells, such as observed with tumor microtube interconnections. As depolarization of normal neural precursor cells during development profoundly affects neural stem cell proliferation, we tested the hypothesis that neuron to glioma synapse-mediated depolarization promotes glioma growth. Using *in vivo* optogenetic techniques to depolarize patient-derived glioma xenografts expressing channelrhodopsin-2 (ChR2), we found that glioma cell depolarization robustly promotes proliferation. Further supporting the functional role of glutamatergic signaling through the AMPA receptor, expression of a dominant-negative AMPA receptor subunit robustly inhibits glioma xenograft growth. These findings define an unexpected integration of glioma cells into neural circuitry, identify excitatory synaptic neurotransmission as a mechanism driving glioma growth and elucidate the previously unexplored potential to target glioma circuit dynamics for therapy of these lethal cancers.

TMIC-10. AUTOPSY STUDY ON THE EFFECTS OF TUMOR TREATMENT FIELDS IN RECURRENT GLIOBLASTOMA: PRELIMINARY RESULTS

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BACKGROUND: Treatment of recurrent glioblastoma using tumor treatment fields (TTFields) has recently been approved. This resulted from a recent clinical trial that showed better quality of life and comparable overall survival. Low-intensity, alternating frequency electric fields, known as TTFields, have been shown to disrupt cell division and subsequently tumor growth. Apoptosis and cell cycle arrest have been seen in vitro, and shown in mice and rabbit tumor models. Glioblastoma patients who have undergone TTField therapy have not yet been assessed at autopsy to determine both the pathological signature of TTField therapy, and the pattern of failure. METHODS: Whole brain samples were acquired and analyzed pathologically from six recurrent GBM patients at autopsy. Three patients served as controls and three were considered test patients who had undergone TTField therapy. Tissue samples were acquired from regions suspicious of tumor and treatment effects. Samples were paraffin embedded and hematoxylin and eosin (H&E) stained, and pathologically reviewed by a board certified pathologist. Pathomic features of interest were extracted from each sample, including texture features and cellular morphometrics. Samples were then compared statistically. RESULTS: Regions of necrosis and cellular debris were more prevalent in the patients who underwent TTField therapy. Some pathomic features of interest differed significantly between the two groups. CONCLUSION: These findings suggest there is increased apoptosis in patients treated with TTFields compared to those on chemoradiation alone. Recruitment is ongoing for expansion of this study.

TMIC-12. TUMOR-HOMING RNA-NANOPARTICLES REPROGRAM IMMUNE CELLS IN THE BRAIN TUMOR MICROENVIRONMENT

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BACKGROUND: Brain tumors are particularly difficult to treat due to their relative isolation behind the blood brain barrier. Cytotoxic T cells elicited by cancer vaccines are capable of penetrating this barrier, but are limited by innate immune cells in tumor microenvironments that inhibit T cell function. There is therefore an unmet need for a method to reprogram the immune cells in the tumor microenvironment to promote antitumor T cell responses. OBJECTIVE: We previously reported that systemically administered liposomes bearing RNA encoding tumor antigens profoundly activate innate immune cells in reticuloendothelial system (RES) organs. Here, we report a modified liposome formulation capable of redirecting this immunomodulatory nucleic acid cargo to immune cells in brain tumors. APPROACH: Cationic liposomes with varying compositions were loaded with Cy3-labelled RNA or siRNA and injected intravenously into glioma-bearing mice. RNA uptake was assessed with flow cytometry and immunofluorescence microscopy after 18 hours. RESULTS: Inclusion of cholesterol within liposomes increased mRNA uptake in intracranial GL261 and KR158b tumors after systemic injection in a dose-dependent manner. Optimized tumor-homing liposomes delivered mRNA encoding tumor antigens to CD45+ immune cells in both tumors. These liposomes were also used to deliver siRNA against programmed death ligand 1 (PDL1). siRNA-loaded liposomes reduced PDL1 expression on dendritic cells *ex vivo* and on transfected CD45+ cells in murine brain tumors. CONCLUSIONS: Our optimized liposomes effectively deliver mRNA and siRNA to immune cells in multiple murine brain tumors. Future work will consider the mechanism of this enhanced delivery and the use of tumor-homing liposomes to deliver other immunomodulatory biomolecules.

TMIC-13. EFFICACY OF RETINOIC ACID IN REVERSING IMMUNE EVASION IN IDH MUTANT GLIOMAS

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BACKGROUND: Most diffuse gliomas are characterized by mutations in isocitrate dehydrogenase 1 or 2 (IDH1/2). Among its many effects, these mutations drive specific epigenetic programs that result in tumor cell intrinsic resistance to innate lymphoid cells. Here, we define a central role for retinoic acid signaling in mediating immune resistance to innate anti-tumor immunity in IDH mutant tumors. IDH mutations in gliomas suppress retinoic acid signaling through transcriptional repression of Retinol Binding Protein 1 (RBP1), a key chaperone protein in retinoic acid biosynthesis. Loss of retinoic acid signaling results in diminished expression of retinoic-acid-responsive genes, including activating NKG2D ligands ULBP1 and ULBP3 and results in resistance to natural killer (NK cells). METHODS: We evaluated the efficacy of all-trans retinoic acid (ATRA) as a therapeutic adjuvant for IDH mutant gliomas. In a series of in vitro and in vivo experiments, we assessed the ability of ATRA to increase NKG2D ligand expression in IDH mutant cells, and its ability to increase NK-mediated recognition and killing. We also looked at the impact of ATRA on the tumor immune microenvironment and its underlying mechanisms RESULTS: ATRA treatment led to increased recognition and killing of IDH mutant glioma cells by NK cells, by increasing NKG2D ligand expression. In vivo, ATRA treatment led to significantly slower tumor growth in IDH mutant tumor-bearing mice. Importantly, we made the unexpected finding that restoration of retinoic acid signaling dramatically reshapes the landscape of the immune microenvironment by permitting infiltration of NK cells and CD8 T cells in a manner dependent on the chemokine CCL2. CONCLUSIONS: Retinoic acid therapy using ATRA results in marked suppression of IDH mutant glioma tumor growth in an NK cell-dependent fashion in preclinical animal models of glioma. These findings have important therapeutic implications for the treatment of IDH mutant diffuse glioma.

TMIC-14. AUTO-/PARACRINE SIGNALING OF PI3K/AKT/YKL-40 IN MESENCHYMAL GLIOBLASTOMA PROGRESSION

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Glioblastoma multiforme (GBM), the most common and deadliest brain tumor with a median survival of 12–15 months, has been characterized by robust angiogenesis, high invasiveness, and universal recurrence. Genomics and transcriptomics studies defined three GBM subtypes (proneuronal, classical, mesenchymal), which are associated with genomic abnormalities, treatment response, and diversity in tumor microenvironment. However, molecular mechanisms of subtype-specific genotype in contribution to tumor phenotype are less understood. We have previously generated a *de novo* human GBM model by inactivation of p53 and activation of AKT signaling pathways in human neural stem/progenitor cells (hNSCs). Further characterization of this model indicates that the tumors recapitulate GBM's classical histopathogical features and exhibit a molecular profile resembling the mesenchymal subtype. Comparative transcriptomic analysis revealed that YKL-40/CHI3L1, a secreted glycoprotein, is significantly upregulated during AKT activation induced malignant transformation of hNSCs *in vitro* and *in vivo*. Pharmacological inhibition of PI3K/AKT/mTOR signaling pathway results in decreasing YKL-40 mRNA expression and protein secretion level. Mechanistically, motif enrichment analysis and functional validation revealed that YKL-40 is bidirectionally regulated by a gene regulatory network with two transcription factors, BACH2 and CEBPB. Furthermore, YKL-40 significantly activates AKT-S6 and MAPK-ERK kinase signaling pathways, resulting in enhancing tumor cells proliferation, neurosphere and soft-agar colony formation, and tumor progression. *In vivo*

silencing YKL-40 attenuates tumor angiogenesis and prolongs animal survival in the orthotopic xenograft mouse models. Interestingly, knocking down YKL-40 expression decreases microglia/macrophages infiltration in tumor mass. Both *in vitro* and *in vivo* validation indicate that PI3K/AKT/YKL-40 mediates tumor-associated microglia/macrophages recruitment and activation during tumor progression. Taken together, these results suggest that auto- and paracrine signaling of PI3K/AKT/YKL-40 affect tumor cells and tumor microenvironment contributing to glioblastoma progression, indicating YKL-40 as a predictive biomarker for evaluation of anti- PI3K/AKT/mTOR therapy, and a potential drug target for the treatment of this devastating disease.

TMIC-15. OMX IS A TUMOR MICROENVIRONMENT MODIFIER THAT RESTORES ANTI-TUMOR IMMUNITY AND IMPROVES ANTI-TUMOR EFFICACY BY REDUCING TUMOR HYPOXIA IN INTRACRANIAL GLIOBLASTOMA MOUSE MODEL

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BACKGROUND: Intratumoral hypoxia is associated with resistance to chemo- and radio-therapies and poor patient outcomes. In addition, hypoxia promotes the immune escape of tumors by altering the recruitment and function of innate and adaptive immune effector and suppressor cells. Therefore, reversing tumor hypoxia to create an immunopermissive microenvironment may improve anti-tumor response, and combined with immunotherapy approaches such as checkpoint inhibitors (CPI) may increase therapeutic efficacy. OMX, an anti-cancer therapy designed to reverse tumor hypoxia, efficiently accumulates in orthotopic rodent GB and spontaneous canine brain tumors, reduces tumor hypoxia and enhances immunotherapeutic efficacy. METHODS: We used in vivo bioluminescence imaging of tumor, immunohistochemistry, flow cytometry, and cytokine multiplex assays to evaluate OMX's ability to immunosensitize the GL261 brain tumor microenvironment and promote tumor cures. RESULTS: Following intravenous administration in brain tumor-bearing mice, OMX reduces tumor hypoxia, modulates the IFNg signaling pathway, enhances the infiltration of tumor-specific CX3CR1^{+} CD8 T cells into the tumor (using the EphA2 as a GL261-specific tumor antigen), increases the activation of cytotoxic T lymphocytes (CTLs), decreases Tim3 and Lag3 exhaustion markers on CD8 T cells, and reduces the number of immunosuppressive cells such as MDSCs and Tregs in the tumor. Similar immunological changes are observed when OMX is combined with anti-PD-1. In late-stage tumor-bearing mice, we observed a 40% tumor cure rate for the combination of OMX with anti-PD-1, while anti-PD-1 alone resulted only in 5% tumor cures. Following rechallenge with GL261 tumor cells injected on the other side of the brain, all mice treated with the combination of OMX with anti-PD-1 survived, indicating the presence of long-term immunological memory against glioma cells. CONCLUSION: By delivering oxygen specifically to the hypoxic tumor microenvironment, OMX may restore anti-cancer immune responses in GB patients and synergize with radiotherapy and immunotherapy to enhance tumor control and improve patient outcomes.

TMIC-16. CORE-LIKE TUMOR CELLS PROMOTE MALIGNANCE OF GLIOBLASTOMA VIA INTERCELLULAR CROSSTALK WITH EDGE-LIKE TUMOR CELLS IN A HDAC1-CD109 DEPENDENT MANNER

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Glioblastoma (GBM) is a malignant primary brain cancer without cure, due to the edge infiltrating tumor cells and non-complete resection. Although intratumoral heterogeneity is recognized in various cancers including GBM, its spatial distribution and intercellular crosstalk remain poorly understood. Previously, we identified two mutually exclusive glioma stem-like cells (GSCs) subtypes in GBM, termed mesenchymal (MES) and non-MES GSCs. Here, we tested the hypothesis that non-MES and MES GSCs have inter-cellular crosstalk to promote GBM aggressiveness and heterogeneity. We found that MES GSCs are preferentially located in the tumor core region, while non-MES GSCs subside in the tumor edge. Co-culture with MES GSC-containing cultures (glioma spheres) promoted both in vitro growth and in vivo tumorigenicity of non-MES ones. By a high throughput screening, several HDAC inhibitors selectively eliminated MES glioma spheres, while preserving non-MES glioma spheres. Among the HDAC family, HDAC1 is upregulated in GBM and associated with unfavorable outcome. Knockdown of HDAC1 blocked the intercellular pro-tumorigenic signal from MES glioma spheres to the non-MES counterparts. Mechanistically, CD109 is secreted from MES glioma spheres, thereby contributing intercellular pro-tumorigenic signals from MES to non-MES spheres. By RNA sequence, we found that HDAC1 regulates transcriptional activity of CD109 via binding to the promoter of CD109 together with the MES-specific transcription factor C/EBPb. To validate the intercellular signals in a single tumor, we established 6 clones of spheres from both tumor core and edge derived from the same GBM patient. The core lines contained those with higher resistance to irradiation (IR) treatment in comparison to the edge-derived counterparts. Furthermore, the core glioma spheres that showed higher MES signature promoted the growth and transformation of the non-MES like edge-derived glioma spheres both in vitro and in vivo. Collectively, our data identified HDAC1-CD109 dependent intercellular crosstalk from core like MES GCSs to edge like non-MES GSCs.

TMIC-17. IMMUNE MICROENVIRONMENT OF NF2-ALTERED RADIATION-INDUCED MENINGIOMAS

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INTRODUCTION: Radiation-induced meningiomas (RIMS) are more clinically aggressive than their sporadic counterparts, with higher rates of tumor recurrence, multiplicity and neurological impairment. Our laboratory has previously demonstrated that RIMs have a distinct genomic profile with a subset of tumors harboring NF2 inactivation through genomic rearrangements. In this study we establish the clinical characteristics and immune microenvironment between RIMs with and without the NF2 alterations. METHODS: A retrospective chart review was performed on 31 RIMs. Volumetric analysis was performed using ITK-SNAP on serial preoperative imaging to estimate tumor growth rate. Immunohistochemistry staining for PD-1, PD-L1, CD-3, CD-163, and FOX-P3 were performed. Results were correlated with whole exome sequencing and RNA sequencing data previously published. Pathway analysis was performed using Gene Set Enrichment Analysis (GSEA) on RNA sequencing data using the differentially expressed genes. RESULTS: Clinically, NF2-altered tumors and NF2-WT (wildtype) tumors did not differ based on age at diagnosis, indication for previous radiation, WHO grade, histopathological subtype, extent of resection and tumor location. NF2-altered tumors had a significantly faster growth rate compared to NF2-WT tumors (3.8X faster, $p>0.05$). The difference in growth rate was independent of tumor grade. PD-L1 staining was negative on tumor cells in NF2-altered tumors and patchy positivity in 60% of NF2-WT tumors ($p < 0.05$). In contrast, 100% and 60% of tumors had PD-L1 positive immune cells in NF2-altered and NF2-WT tumors, respectively ($p<0.05$). The degree of CD3+ lymphocyte infiltration was lower in NF2-altered tumors ($t=2.73$, $p<0.05$). CONCLUSIONS: Our findings suggest that there is a difference in the immune microenvironment between NF2-altered and NF2-WT RIMs. NF2-altered tumors have a lower degree of CD3+ lymphocyte infiltration and higher frequency of PD-L1 positive immune cells that are indicative of an immunosuppressed microenvironment. Further work is needed to elucidate pathways that may be targeted to manipulate the immune microenvironment.

TMIC-18. IMPACT OF HFE MUTATION ON VIABILITY IN MACROPHAGES EXPOSED TO GLIOBLASTOMA EXOSOMES

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In Glioblastoma (GBM), the tumor microenvironment is critical to cancer development and progression. Glioblastoma Extracellular Vesicles (GBM-EVs) have been shown to promote a tumor-supporting phenotype in macrophages, leading to surrounding immunosuppression and enhanced tumor growth. Microglia and macrophages contribute to iron homeostasis, essential for cellular energy production in rapidly dividing cells. The HFE mutation is associated with altered cellular iron homeostasis. It is the most common autosomal recessive polymorphism found in Caucasians and may be linked to worse patient prognosis in GBM. Our lab showed that HFE mutation confers phenotypic differences between HFE and wild-type (WT) macrophages, with HFE macrophages displaying increased migration and

phagocytosis. How these phenotypic differences may present in response to GBM exosomes has not yet been explored. Primary macrophage cultures isolated from bone marrow of WT and HFE mutant knock-in mice were exposed to exosomes from different patient-derived cancer stem cells. Significant differences in macrophage viability 24 hours after exosome exposure were observed in response to GBM exosomes (p= 0.001). When exposed to GBM exosomes, WT macrophage viability consistently decreased while the viability of macrophages with the HFE mutation increased. There was no significant difference between the two genotype macrophage groups when exposed to U87 exosomes, supporting that the stem cells are modulating macrophage phenotype. These data suggest that genotypic differences in iron handling pay a critical role in macrophage response to GBM exosomes and may be responsible for differences seen in disease severity in patients with HFE mutations.

TMIC-19. USING QUANTITATIVE MR IMAGING TO RELATE GBM MASS EFFECT TO PERFUSION AND DIFFUSION CHARACTERISTICS OF THE TUMOR MICRO-ENVIRONMENT

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Biomechanical forces are known to affect tumor growth and evolution [1]. Likewise, tumor growth drives physical changes in the micro-environment that affect tissue solid and fluid mechanics. Tumor mass effect, resulting from rapid tumor cell proliferation, has been shown to be prognostic for poor outcome in glioblastoma (GBM) patients and to be associated with the expression of gene signatures consistent with proliferative growth phenotype [2]. Similarly, elevated interstitial fluid flow (IFF) has been shown to drive GBM invasion [3]. This study investigates the relationship between tumor mass effect, diffusion, perfusion and IFF in GBM using anatomical (pre- and post-contrast T1 weighted, T2/FLAIR) and quantitative MR imaging (Dynamic Contrast Enhanced (DCE) MRI, and Diffusion Weighted Imaging (DWI)). We use data from 39 patients from the Ivy Glioblastoma Atlas Project (Ivy GAP)[4] which provides matched imaging, ISH, RNA, gene expression and clinical data over the course of treatment. We analyze pre-operative anatomic imaging data to determine the tumor-induced mass effect in each patient using quantitative measures such as 'Lateral ventricle displacement' [2]. Perfusion and diffusion measures are derived from pre-operative DCE and DWI imaging. Additionally, we estimate IFF velocities in the tumor region using DCE imaging data in combination with a computational model of fluid flow [5]. We will report the results of quantitative imaging analysis in relation to tumor mass effect and examine correlations with biological tumor characteristics and treatment outcome. We further investigate our findings in patient-derived xenograft models of GBM. References: [1] R.K. Jain et al. Annu. Rev. Biomed. Eng., 2014, 16, 321–346. [2] T.C. Steed et al. Scientific Reports, 2018, 8, 2827. [3] K.M. Kingsmore et al. Integr. Biol., 2016, 8 1246-1260 [4] N. Shah et al. Data from Ivy GAP. The Cancer Imaging Archive 2016. [5] K.M. Kingsmore et al. APL Bioengineering (In press).

TMIC-20. INTERSTITIAL FLUID FLOW MAGNITUDES DRIVE CHANGES TO THE GLIOMA MICROENVIRONMENT

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Interstitial fluid flow around brain tumors is increased above normal levels. This force has been shown to drive tumor cell invasion in brain tumors and alter the extracellular matrix, vasculature, immune and cellular niches in non-nervous tissues in ways that promote tumor progression [1]. Here, by applying our dynamic contrast enhanced MRI method and analysis [2], we measured interstitial fluid flow in multiple patient cell line-derived xenograft models of glioma in mice (n=6–8 per group, four different lines). The range of interstitial velocity magnitudes is similar across these models (range from 0.1–2.6 micron/s), though intratumoral variability is high. We examined tumors by immunohistochemistry including tumor cells (proliferating cells, ki67; stem-like cells, Nestin and Sox2; apoptotic cells, Caspase 3/7), the cellular microenvironment (astrocytes, GFAP and GLAST; microglia/macrophages, Iba-1 and CX3CR1; blood vessels, CD31; neurons, NeuN and Neurofilament; oligodendrocytes, OSP1), and of the extracellular space (hyaluronan, HABP; tenascin C; white matter tracts, fluoromyelin; cytokines). Fluid flow pathways and rates are dictated by anatomical features of the brain in these models (i.e. blood vessels and white matter tracts). Blood vessel density did not significantly correlate with regions of higher velocities. Based on our observations we performed in vitro experiments in a 3D tissue engineered model of the glioma microenvironment [3] to assess invasion within the range of measured flow rates. Velocities were controlled using either microfluidic pump driven flow or pressure driven flow and cellular outcomes were assessed using flow cytometry and immunocytochemistry of the 3D gel system. The results of these studies will be discussed. 1) Munson, Shieh, *Cancer Management Research* (2014) 6: 317; 2) Kingsmore, Vaccari, Abler, et al. *APL Bioengineering* (*In press*); 3) Harris, Yuan, Munson, *Methods* (2018) 134:20–31.

TMIC-21. THE POTENTIAL CONTRIBUTION OF PERICYTES TO GLIOBLASTOMA MULTIFORME TUMOUR MICRO-ENVIRONMENT IMMUNOSUPPRESSION VIA DAMPENED EXPRESSION OF ICAM-1, VCAM-1 AND MCP-1

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Glioblastoma Multiforme (GBM) is the most aggressive, fatal, yet most common form of brain malignancy in adults. Despite advances in immune-based treatments for other modes of cancer, GBM remains a challenge due to its ability to dampen immune responses via mechanisms not yet fully understood. With a median survival time of only 15 months following diagnosis, there is a strong push to find new targets for therapy. An emerging aspect of GBM's pathogenesis is the idea of the tumour's immunosuppressive microenvironment which comprises a mixture of malignant tumour cells, stroma, blood vessels and infiltrating inflammatory cells. These cells can alter their phenotypes to express less pro-inflammatory and more anti-inflammatory mediators. Despite advances in understanding the contribution of these cells in establishing an anti-inflammatory microenvironment, the contribution of pericytes, an important neurovascular mural cell that forms the blood-brain barrier, has been inadequately studied. Therefore, we investigated the changes in immune responses between the non-neoplastic brain and GBM-derived pericytes isolated directly from the same patient's tissues obtained during neurosurgery. Our results show that cellular responses to pro-inflammatory stimuli such as Interleukin 1 beta (IL-1ß) were dampened in GBM-derived pericytes when compared to pericytes isolated from non-neoplastic brain tissue from the same patient. More specifically, GBM-derived pericytes showed lower induction of pro-inflammatory molecules, Intracellular Adhesion Molecule 1 (ICAM-1), Vascular Cell Adhesion Molecule (VCAM-1), and Monocyte Chemoattractant Protein 1 (MCP-1) when compared to normal brain pericytes. Due to the involvement of these molecules in immune cell recruitment and infiltration, this decreased response may contribute to the maintenance of GBM microenvironment immunosuppression. This highlights potential targets for alleviating such immunosuppression, which could enhance the success of immunotherapy-based treatments for GBM.

TMIC-22. EXOSOMAL NON-CODING RNAS MEDIATE THE CROSS-TALK OF BRAIN METASTASIS CANCER STEM CELLS AND MICROGLIA

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Brain metastases are the most common secondary brain tumors in adults. Despite their high frequency and patient poor prognosis, very little research has been performed on lung tumor brain metastases, mainly due to the lack of appropriate experimental models. In this study, we isolated cancer stem cells (CSCs) from fresh specimens of lung tumor brain metastases. The CSCs were analyzed for sphere formation and limiting dilution analyses, stemness markers, ability to generate xenografts and for their interaction with microglia cells. We found that CSCs derived from brain metastases had a high sphere forming capacity and self-renewal ability comparable to that of glioma stem cells. The CSCs expressed the stemness markers, CD133, Sox2, Klf4, Aldh2a, CD44 and the lung tumor markers, cytokeratin 7, and CD166. Transplantation of the CSCs or organoids generated from the brain metastases formed xenografts that recapitulated the parental tumors. These xenografts were infiltrated by a large number of amoeboid microglia that expressed high levels of M2 markers. Using co-culture experiments, we further found that CSCs derived from brain metastasis induced the polarization of microglia to the M2 phenotype via secreted exosomes. Similarly, M2 micrglia cells increased the self-renewal and stemness of the CSCs. RNA seq analysis identified specific miRNAs and lncRNAs that were associated with the CSC-microglia interactions. Using specific reporters, antagomiRs and

CRISPR/Cas9 we demonstrated that miR-21, miR-1246 and the lncRNA TALNEC2 played a major role in the M2 polarization of microglia cells both in vitro and in vivo. In conclusion, we generated CSCs and organoids from lung tumor-derived brain metastases and identified potential therapeutic targets for these tumors. The established CSCs can serve as valuable in vitro and in vivo models for analyzing mechanisms involved in brain metastasis, for studying the tumor-microenvironment interactions and for personalized high throughput screening of new and repurposed drugs.

TMIC-23. CHEMOTHERAPY AND TAU INTERPLAY FACILITATES BREAST TO BRAIN METASTASIS

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Improving the treatment of breast to brain metastases (BBM) for women with breast cancer represents a leading edge of treatment failure for Her2+and triple negative disease. Over the past several decades a wide range of tumor-centric studies have identified genes and their regulators within signaling pathways promoting breast cancer metastasis. The current prevailing view which has dominated research focus is that metastatic cancer cells enter the brain by crossing the blood-brain-barrier (BBB). However, other than the popular BBB, the central nervous system has another crucial, yet more permeable barrier: blood-cerebral spinal fluid barrier (BCSFB) made up of choroid plexus (CP). The current study exploits foundations of neuroscience to advance our understanding of the brain and metastatic breast cancer cells through the perspective of the CP/BCSFB --This bidirectional interplay of tumor cells and this native brain cells in metastases remains poorly understood and studied. Many breast cancer patients treated with chemotherapy complain of impaired memory and several basic cognitive dysfunctions before brain metastases diagnosis. Several studies have confirmed the detrimental effects of chemotherapy on cognitive performance, known as "chemo-brain." Furthermore, recent evidence show chemotherapy promotes breast cancer metastasis. The abnormally phosphorylated and aggregated form of tau plays a critical role in neurodegenerative diseases as of such as Alzheimer's Disease (AD). We hypothesize that chemotherapy induces tau expression in primary breast cancer cells leading to BCSFB permeability and BBM. Our results show: 1) an increase in abnormal tau expression in chemo-treated primary breast cancer cells and BBMs, 2) BBM-derived tau is abnormal and forms paired helical filaments (PHFs), similar to AD, and 3) there was a downregulation of the tight junction markers in chemo-treated BCSFB. Our results suggest a mechanism underlying the linkage between chemo-brain, breast to brain metastasis and its subsequent neurodegeneration.

TMIC-24. TUMOR-SUPPRESSIVE AND ANTI-INFLAMMATORY microRNA-93 IS DECREASED IN GLIOBLASTOMA PATIENTS

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INTRODUCTION: Differential expression of microRNAs (miRs) during inflammation and hypoxia is increasingly appreciated as central driver of glioblastoma (GBM) progression. As miR-93 inhibits expression Interleukin(IL)-8, we hypothesized that miR-93 might be a key regulator of inflammation within the GBM microenvironment. METHODS: Stereotactically obtained GBM specimens were analysed by qRT-PCR. U87 cells were transfected with miR-93 by electroporation and subsequently incubated with IL1b in 5% O2 to mimic the GBM microenvironment. Whole transcriptome microarrays were processed and analyzed by bioinformatics. Invasion and angiogenesis were studied using U87 cell migration and endothelial tube formation assays. Expression levels were measured by qRT-PCR, multiplex assays, and SDS-PAGE (HIF1A, MAP3K2). Binding of miR-93 to 3`UTRs was analysed by luciferase reporter assays. Experiments were performed in triplicates at least 4 times. Results are presented as mean±SEM. P-Values were calculated using Student's t-test. RESULTS: GBM specimens (n=33) and primary cell lines (n=5) revealed decreased levels of miR-93 compared to normal brain tissue (-55.6%±14% and -72.2%±22.3% p<0.05). Supernatants of miR-93-transfected U87 cells impaired U87 cell migration and attenuated angiogenesis of endothelial cells (migration:-58%±12.2%; angiogenesis:-62.4%±19.1%, n=5, p<0.05). Transfection of miR-93 resulted in strong repression of inflammatory genes as shown by transcriptome analyses and qRT-PCR (IL6:-89.6%±15.6%; IL8:-86.4%±16.5%; IL1b:-69.8%±13%; LIF:-59.7%±5.,9%, CSF3:-88.8%±17.9%; COX2:-76.3%±14.2%, CXCL5:-74.2%±13.8%, MAP3K2:-39.5%±20.9%; HIF1A:-15.4%±1.5%; n=4, p<0.05). Luciferase activity of five 3`UTR reporters were significantly quenched after miR-transfection (IL6:-29.2%±10.8%; LIF:-22%%±6.5%; CSF3:-31.8%±10.7%, MAP3K2:-45.3%±21.7%; HIF1A:-32.9%±8.4%, n=5, p<0.05), while COX2, CXCL5 exhibited no decrease. Combined knockdown of MAP3K2 and HIF1A significantly reduced COX2- and CXCL5 levels thus proving indirect regulation (-39%±4.3% and -25.9%±7.1%, respectively, n=8, p<0.05). CONCLUSION: These results suggest an anti-inflammatory and tumor suppressive role of miR-93. Through direct targeting of proinflammatory cytokines, MAP3K2, and HIF1A, miR-93 reduces tumor-initiated inflammation and inhibits shaping of a tumorigenic microenvironment. MiR-93 thus might be a promising target in new GBM treatment strategies.

TMIC-25. DISSECTING THE ROLE OF HOST GENETICS IN GLIOMA EVOLUTION USING GENETICALLY-ENGINEERED MOUSE MODELS AND THE COLLABORATIVE CROSS

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Gliomas have fatal outcomes and few effective treatments. Precision medicine promises to target somatic tumor mutations, but the impact of host genetics on glioma biology remains unclear. Germline polymorphisms have been associated with glioma risk, but such studies cannot disambiguate loci affecting cancer cell-autonomous versus tumor microenvironment (TME) phenotypes, since germline variation is shared by both. Here, we implement a novel platform to discover genetic modifiers of both cancer cell autonomous and TME phenotypes using genetically defined non-germline genetically-engineered mouse (nGEM) glioma models and genetically diverse hosts from the Collaborative Cross (CC). We stereotactically injected *Nf1;Trp53*$^{-/-}$ oligodendrocyte progenitor-derived tumor cells into syngeneic C57/BL6 control mice and 14 different CC strains. Survival analysis showed 7 strains with significantly prolonged survival relative to controls (P<0.05), suggesting slower tumor growth (Gs, growth slow). The remaining 7 strains survived similarly to controls, suggesting fast growth (Gf, growth fast). Variable tumor growth in CC mice suggests host genetic background influences molecular processes in the TME that potentiate or inhibit tumor growth. To identify such candidates, we performed RNA sequencing on 36 tumors from 3 Gf strains, 4 Gs strains, and controls. We identified differentially expressed genes that segregated Gf, Gs, and control tumors (P<0.05). Gene ontology analyses showed that these genes were significantly associated with immune response or extracellular matrix biology. These results suggest that Gs strains activate immune and TME processes that slow tumor growth. Quantitative trait locus (QTL) analyses of mouse host genetics and tumor data will facilitate identification of genetic variants that influence tumor progression. Future studies using human datasets are planned for validation of candidate host loci identified in mice.

TMIC-26. MiR-181a CONTROLS THE OSTEOPONTIN-MEDIATED IMMUNE CIRCUIT IN GLIOBLASTOMA

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INTRODUCTION: MiRNAs can silence a broad gene set, which may benefit heterogeneous tumors such as glioblastoma. Osteopontin has been shown to have an oncogenic role in a variety of cancers and may have immune modulatory effects on macrophages. The current study focuses on using miRNAs to target osteopontin in tumor cells and to modulate immune cells to elicit an antitumor effect. METHODS: Genome-wide profiling followed by evaluation of expression levels of miRNAs and osteopontin were measured using quantitative real time PCR and ELISA in mouse and human glioblastoma and macrophages. Luciferase assays were used to determine the binding potential of miRNAs to osteopontin mRNA. miRNA mimics and forced overexpression using lentiviruses were used to target osteopontin in immune competent murine models of glioblastoma. Nano string profiling and Gene Set Enrichment Analysis (GSEA) were conducted to identify differences in biological states. RESULTS: Microarray analysis demonstrated that osteopontin was the most significantly upregulated gene in human glioblastoma-associated infiltrating macrophages that had originated from matched circulating monocytes, and this was validated using qPCR. Based on the 3`UTR sequence of osteopontin, bioinformatics tools identified 5 miRNAs that are conserved and would be able to modulate osteopontin expression. Luciferase assays confirmed that the miR-181 family regulates osteopontin expression. Overexpression of miR-181a in glioblastoma cells led to their decreased proliferation and increased apoptosis *in vitro*. miR-181a treat-

ment of immune competent mice bearing intracranial glioblastoma demonstrated a 22% increase in median survival time relative to control mice (p=0.006). CONCLUSIONS: miR-181a controls osteopontin expression in both glioblastoma and macrophages by regulating their proliferation and apoptosis.

TMIC-27. GLIOMA CELLS INDUCE 'EPIGENETIC MEMORY' IN MICROGLIA AND BLOCK INFLAMMATORY GENE EXPRESSION-IN VITRO AND IN VIVO FINDINGS

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Macrophages are polarized to perform dedicated functions by microenvironmental signals and upon re-stimulation display a memory. Macrophages could be trained to respond to a specific signal, and upon re-stimulation display a "memory" of a prior insult which could be relatively stable. A subsequent stimulus evokes lesser reaction or is unable to induce a reaction comparable to primary one. Microglia (brain resident macrophages) and peripheral macrophages accumulate in malignant gliomas, and acquire the immunosuppressive, tumor supporting phenotype. This phenotype is difficult to overcome and could be a big obstacle in reestablishing antitumor immunity. Here, we demonstrate that microglia pre-exposed to glioma attain an "epigenetic memory" and display reduced inflammatory gene expression after stimulation with lipopolysaccharide (LPS). We studied if those changes rely on DNA methylation and histone modifications. We found that unstimulated microglia have unmethylated DNA and active histone marks at selected gene promoters indicating chromatin accessibility. Stimulation of cultured microglia with glioma conditioned medium (GCM) or LPS induces distinctive changes of histone modifications: histone acetylation is erased at tested genes due to increased activity of histone deacetylases (Hdac) only in glioma-activated microglia. Inflammatory genes acquire repressive histone marks (H3K27 trimethylation) at later times in a process of epigenetic memory formation. Hdac inhibitors block glioma-induced changes in histone modifications and restored cell ability to linduce effective inflammatory gene expression. Microglia (CD11b+ cells) isolated by flow cytometry from rat brains implanted with C6 glioma cells exhibit changes in activating and repressive marks at the promoters of the tested genes: *Cxcr1, Id1, Id3, Mark1, Zbp1, Irf7* and *iNOS* in comparison to control microglia. Our study reveals important contribution of epigenetic mechanisms to glioma-induced polarization of tumor infiltrating microglia towards a tumor-supportive phenotype. This study was supported by HOMING PLUS/2011–3/7 grant from Foundation for Polish Science (MM).

TMIC-28. GLIOBLASTOMA EXPLOITS CELL SURFACE GLYCOSYLATION-MEDIATED IMMUNE REGULATORY CIRCUITS FOR IMMUNE ESCAPE

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Glioblastoma is the most aggressive brain malignancy, for which conventional therapy has failed to achieve major improvements in survival since 2005. Glioblastoma-associated immune infiltrates are dominated by tumour-associated macrophages (TAM), which could be the key mediators of immune suppression. Recently we proposed the tumour "glyco-code" as a novel target for immunotherapy, and source for biomarkers. Malignant transformation is associated with aberrant expression of cell surface glycans, which play crucial roles in processes such as migration, angiogenesis and immune modulation. Here we investigated the glioblastoma glycocalyx as a tumour-intrinsic immune suppressive mechanism via tumour infiltrating macrophages. We detected increased expression of tumour-associated truncated O-linked glycans in patient-derived glioblastoma- versus control tissues by immunofluorescence and ELISA based assays. In concert, the macrophage galactose lectin (MGL) receptor, which interacts with truncated O-linked glycans, was overexpressed on glioblastoma infiltrating TAM, also detected by immunofluorescence, and confirmed on RNA level in TCGA data. Unsupervised, high-dimensional mass cytometry (CyTOF) analysis of a murine immunocompetent orthotopic glioblastoma model overexpressing truncated O-linked glycans revealed increased tumor infiltration by CCR2+, PD-L1+ macrophages. At a systemic level, we discovered increased frequencies of CCR2+ peripheral dendritic cells (pDC), and a decrease in CCR2+ monocyte frequencies in the bone marrow of these mice. Moreover, we observed a drastic increase in correlations between glioblastoma infiltrating CCR2+ macrophages and several myeloid cell subsets in the bone marrow using network analysis of frequency correlation statistics. Our results suggest that glioblastomas overexpress truncated O-linked glycans and exploit cell surface glycosylation- mediated immune regulatory circuits for systemic immune modulation and recruitment of suppressive TAM.

TMIC-29. REPROGRAMMING BONE MARROW OF TUMOR-BEARING HOSTS FOR GLIOMA IMMUNOTHERAPY

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INTRODUCTION: We recently demonstrated that hematopoietic stem and progenitor cell (HSC) transfer during adoptive T cell immunotherapy (ACT) for malignant brain tumors facilitates extended survival and long-term cures. HSCs migrated to tumors and used T cell-released IFN-g to differentiate into dendritic cells (DCs). In those studies, we transferred syngeneic HSCs from naïve hosts that were not endemic to the tumor-bearing hosts, unlike the clinical paradigm of autologous transfers. In peripheral cancers, HSCs of tumor-bearing hosts (TB HSCs) possess considerable immunosuppressive potential. OBJECTIVE: We therefore evaluated the immunologic function of HSCs from naïve and tumor-bearing hosts to determine if malignant gliomas affect the immune-potentiating function of HSCs. METHODS: We used treatment-resistant intracranial gliomas KR158B and GL261 to generate TB HSCs that were isolated from the bone marrow. We then performed *in vitro* HSC culture experiments alone and with activated T cell supernatants to study HSC differentiation by flow cytometry. Intravenous transfers were used to study HSC differentiation in brain tumors as well as survival benefit during ACT. RESULTS: Culturing naïve or TB HSCs led to differentiation into 30% or 60% myeloid-derived suppressor cells (MDSCs; $CD11b^{+}Ly\text{-}6G/6C^{+}$), respectively. However, when cultured in activated T cell supernatants containing IFN-g, both HSC types differentiated into 30% MDSCs and 80% $MHCII^{+}$ antigen-presenting cells. We then determined that TB HSCs express more IFN-gR than naïve HSCs including 40% more IFN-gR1 and 90% more IFN-gR2 on DC progenitors. *In vivo*, ACT rescued intratumoral TB HSC differentiation, prolonged median survival, and led to long-term cures. CONCLUSIONS: While gliomas exert an immunosuppressive pressure on TB HSCs, ACT can reprogram TB HSC differentiation into functional antigen-presenting cells. A phase I trial evaluating the impact of HSC transfer on adoptive immunotherapy in pediatric high-grade gliomas is underway at our center (ACTION; clinicaltrials.gov NCT03334305).

TMIC-30. COMPUTATIONAL CHARACTERIZATION OF SUPPRESSIVE IMMUNE MICROENVIRONMENTS IN GLIOBLASTOMA

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The immunosuppressive microenvironment in glioblastoma (GBM) prevents efficient antitumoral immune response and thus enables tumor formation and growth. An understanding of the nature of immunosuppression is still largely lacking although it is important for successful cancer treatment through immune system modulation. To gain insight into immunosuppression in GBM, we performed a computational analysis to model the relative immune cell content and type of immune response in each GBM tumor sample from the Cancer Genome Atlas RNA-seq dataset. As a result, we uncovered high variability in immune system-related responses and in the composition of microenvironment across the cohort, suggesting immunological diversity. Immune cell compositions were associated with typical alterations, such as IDH mutation or inactivating NF1 mutation/deletion. Furthermore, our analysis identified three GBM subgroups presenting different adaptive immune responses: Negative, Humoral, and Cellular-like. These subgroups were linked to transcriptional GBM subtypes and typical genetic alterations. Interestingly, all the G-CIMP and IDH mutated samples were in the Negative group, which was also enriched by cases with focal amplification of CDK4 and MARCH9. Overall, our analysis revealed heterogeneity in the immune microenvironment of GBM and identified MARCH9 amplification as a possible new marker for immunosuppression. Characterization of diverse immune responses will facilitate patient stratification improving personalized immunotherapy in the future.

TMIC-31. GENETIC DRIVER-MUTATIONS DEFINE COMPOSITION AND PROPERTIES OF TUMOR-ASSOCIATED MYELOID CELLS IN GLIOBLASTOMA

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The GBM microenvironment is composed of numerous non-neoplastic cells, among which tumor-associated myeloid cells (TAMs) are the most abundant. TAMs are constituted of several different cell types, including brain microglia and bone-marrow-derived infiltrating cells, which can be further

divided into inflammatory monocytes, monocyte-derived macrophages, and polymorphnuclear neutrophils. These myeloid cells are believed to promote tumor growth and immune evasion by expressing immune checkpoint proteins. Remarkable progress has been made in treating lung cancer and melanoma by using immune-checkpoint inhibitors; however, mixed results were obtained for its application in GBM. To examine how molecular-subtypes of GBM influence the composition and immunosuppression of tumor-associated myeloid cells, we used the RCAS/tv-a system, a somatic cell-specific gene transfer system, to generate de novo murine GBM (mGBM). By manipulating different known human oncogenic-driver mutations such as overexpressing PDGFB or EGFRVIII, or silencing NF1, we generated Proneural (PN), Classical (CL) and Mesenchymal (MES) GBMs respectively, which phenotypically resemble their human counterparts. We found that the majority (80%) of tumor-infiltrating myeloid cells in PN GBM are bone-marrow derived macrophages, whereas this number is significantly less in CL and MES mGBM. Interestingly, unique to MES, there is a significant presence (16%) of neutrophils in these tumors than in other subtypes (4%). Further characterization for subtype-specific expression of PD-L1, a druggable immune checkpoint molecule, by flow cytometry shows that MES has the highest expression of PD-L1 (Mean Fluorescent Intensity=13,000 ± 3,000), whereas the expression is lower (MFI=8,100 ± 2,000) in PN and lowest (MFI=5,900 ± 2,000) in CL. The current effort is devoted to examining how myeloid cells isolated from different subtypes can differentially suppress T cell functions; and how mice carry these subtypes respond to anti-PD-L1 therapy. Our findings illuminate the unique composition and functions of tumor-associated myeloid cells in different GBM subtypes, establishing a rationale to target these cells in this heterogeneous neoplasm.

TMIC-32. INHIBITION OF MerTK MODULATES GLIOMA-ASSOCIATED MACROPHAGES AND MICROGLIA IN TUMOR MICROENVIRONMENT

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BACKGROUND: Glioma-associated macrophages and microglia (GAMs) are predominant immune cells in a glioma microenvironment. Receptor tyrosine kinase MerTK is highly expressed in GAMs. Activation of MerTK triggers efferocytosis and polarizes GAMs to an immunosuppressive phenotype, promoting tumor growth. We hypothesize that inhibiting MerTK by UNC2371, a small molecule MerTK inhibitor, may lead to a less immunosuppressive glioma microenvironment. METHODS: Monocytes were isolated from the blood of healthy human donors followed by differentiation and stimulation into proinflammatory ($CD80^+$/$CD86^+$) and anti-inflammatory ($CD163^+$/$CD206^+$) macrophages by granulocyte-macrophage colony-stimulating factor (GM-CSF)/Interferon-gamma and macrophage colony-stimulating factor (M-CSF)/Interleukin-4 respectively. Phenotyping of the macrophages was done by flow cytometry analysis of the cell surface markers. Cytotoxicity of UNC2371 in GBM cells and macrophages was determined by cell viability. Cytokine profiling from the macrophages and a human microglial cell line was performed using cytokine array analysis. The protein expressions of arginase I, interleukin-8, and MerTK were quantified by Western blotting. RESULTS: We demonstrate that UNC2371 inhibits the growth of GBM cells with EC50 of 95.5 nM in U251 glioma cells, but not macrophages. While cultured in U251 conditioned medium (CM), a decrease in $CD163^+$/$CD206^+$(anti-inflammatory) macrophages was observed in 43% of healthy donors (n=7) after UNC2371 treatment, suggesting a proinflammatory polarization. UNC2371-treated macrophages or microglia reduces secretion of Interleukin-8, a chemokine known to promote gliomagenesis, was detected by cytokine array analysis. UNC2371 decreases protein expression of MerTK, Interleukin-8, and arginase I in GAMs. These data suggest that UNC2371 inhibits MerTK signaling in GAMs and alleviates immunosuppression in the glioma microenvironment. CONCLUSION: Our findings suggest that UNC2371 is a promising drug to polarize GAMs towards proinflammatory, suppress pro-glioma cytokines and modulate the glioma microenvironment. Preclinical evaluations of UNC2371 as a tumor microenvironment modulator using animal models are currently underway.

TMIC-33. THE ROLE OF FIBRINOGEN-LIKE PROTEIN 2 ON IMMUNOSUPPRESSION AND MALIGNANT PROGRESSION IN GLIOMA

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BACKGROUND: Virtually all low-grade gliomas (LGG) will progress to high-grade gliomas (HGG), including glioblastoma, the most common malignant primary brain tumor in adults. A key regulator of immunosuppression, Fibrinogen-like Protein 2 (FGL2), may play an important role in the malignant transformation of LGG to HGG. We sought to determine the mechanism of FGL2 on tumor progression and to show that inhibiting FGL2 expression had a therapeutic effect. METHODS: We analyzed human gliomas that had progressed from low- to high-grade for FGL2 expression. We modeled FGL2 overexpression in an immunocompetent genetically-engineered mouse model to determine its effect on tumor progression. Tumors and their associated microenvironment were analyzed for their immune cell infiltration. Mice were treated with an FGL2 antibody to determine a therapeutic effect. RESULTS: We identified increased expression of FGL2 in surgically-resected tumors that progressed from low- to high-grade (N=10). TCGA data showed that LGG cases with over-expression of FGL2 (N=195) had statistically significantly shorter survival compared to cases with low-expression (N=325) (log-rank test, $p<0.001$). In a murine glioma model, HGGs induced with FGL2 exhibited a mesenchymal phenotype and increased $CD4^+$ forkhead box P3 $(FoxP3)^+$ Treg cells implicating immunosuppression as a mechanism for tumor progression. Macrophages in these tumors were skewed toward the immunosuppressive M2 phenotype. Depletion of Treg cells with anti-FGL2 statistically significantly prolonged survival in mice compared to controls (N=11 per group, log-rank test, $p=0.004$), shifted the phenotype from mesenchymal HGG to proneural LGG, and decreased M2 macrophage skewing. CONCLUSION: FGL2 facilitates glioma progression from low- to high-grade. Suppressing FGL2 expression holds therapeutic promise for halting malignant transformation in glioma by decreasing Tregs and polarizing macrophages away from the M2 phenotype.

TMIC-34. Na/H EXCHANGER ISOFORM 1 (NHE1) IN IMMUNOSUPPRESSIVE TUMOR MICROENVIRONMENT IN MOUSE SYNGENEIC GLIOMA MODEL

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Glioblastoma is the most common and aggressive brain tumor with a median survival of ~15 months. Clinical trials of patients for immunotherapy showed an inferior response, which can in part attribute to an immunosuppressive, acidic tumor microenvironment (TME) composed of glioma-associated microglia/macrophages (TAMs), regulatory T-cells (Treg), and expression of immune checkpoint molecules. NHE1 maintains alkaline pHi in glioma and TAMs, promoting their migration and proliferation. Temozolomide (TMZ) therapy concurrently induces upregulation of NHE1 in glioma and alters TME immunogenicity. In this study, we investigated the impact of selective deletion of *Nhe1* from microglia/macrophage using $Cx3Cr1^{CreEr+/-}$;$Nhe1^{f/f}$ mice (*Nhe1* KO). Non-immunogenic SB28-GFP glioma cells were transplanted intracranially, and tamoxifen (75 mg/kg, i.p.) was administered at 1–5 days post-glioma injection (d.p.i.). $Cx3Cr1^{CreEr-/-}$; $Nhe1^{f/f}$ mice receiving identical treatment served as controls (*Con*). These mice were subsequently treated with DMSO or TMZ (2.5 mg/kg, i.p.) at 2–6 d.p.i. TAM infiltration and T-cell profile were analyzed by flow-cytometry at 10 d.p.i. TMZ monotherapy in *Con* mice elevated the tumor infiltrated microglia by 2-fold and macrophage numbers by 2.5-fold. In contrast, *Nhe1* KO mice treated with TMZ did not exhibit a significant increase of microglia or macrophage infiltration. $Ym\text{-}1^+$ tolerogenic macrophages in tumors from TMZ-treated *Nhe1* KO mice were nearly abolished. TMZ treatment in *Nhe1* KO mice also reduced the tumor-promoting $CD4^+CD25^+FoxP3^+$ Treg infiltration but did not change $CD8^+GZMb^+$ and $CD8^+IFN\gamma^+$ populations. Moreover, combining TMZ with selective deletion of *Nhe1* in $Cx3Cr1^+$ cells significantly elevated $CD4^+PD\text{-}1^+$ and $CD8^+PD\text{-}1^+$ cell population. Most importantly, *Nhe1 KO* mice treated with combinatorial TMZ and anti-PD-1 therapy prolonged the animal survival. Taken together, our study suggests that NHE1 is involved in modulating TME and PD-1 expression. Blockade of NHE1 function in combination with TMZ and anti-PD-1 could improve TME and provide an alternative treatment strategy for glioblastoma. This was supported by NIH-grant R01NS75995(DS).

TMIC-35. ASTROCYTE-DEPENDENT ENHANCEMENT OF GLIOBLASTOMA GROWTH AS A CANDIDATE THERAPEUTIC TARGET

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Glioblastoma multiforme (GBM) is a disease with an urgent need for deeper understanding and new therapeutic approaches. As in other solid

tumors, the tumor microenvironment (TME) is likely to control growth and sensitivity to treatment. Key components of the TME of GBM include the vasculature, microglia and astrocytes. Whereas GBM vasculature and microglia have been extensively studied, less is known about the potential involvement of astrocytes in GBM biology. In this study, astrocytes were investigated with regard to their effect on glioblastoma growth through in vitro approaches and in an in vivo model. Our studies, using different established GBM cell lines, and patient-derived primary GBM cultures, show that co-cultured astrocytes increase GBM cell number by supporting GBM cell proliferation. Furthermore, orthotopic co-injection of astrocytes and GBM cells in mice leads to reduced survival as compared to GBM cell mono-injection. Gene-expression analyses of mono- or co-cultured astrocytes have identified a GBM-cell-induced astrocyte gene signature. Analyses of two publically available GBM gene expression data set demonstrated that the signature of "GBM-educated" astrocytes was associated with significantly shorter survival. These analyses also indicated differences between molecular subgroups of GBM regarding the signature, such that the mesenchymal subset displayed a higher signature score as compared to the other molecular GBM subtypes. A high-throughput screening of approximately 1200 compounds of the "Prestwick-library" has been performed to identify compounds which specifically block the astrocyte-dependent proliferation of U343MG GBM cells. Following the initial screen, 12 compounds have been validated as specifically acting on GBM growth in co-culture. Two of them have been selected for further studies. In summary, this ongoing study suggests that astrocytes contribute to glioblastoma growth and implies this crosstalk as a candidate target for novel therapies. Clinical relevance of the model system is suggested by the prognostic significance of the signature of GBM-educated astrocytes.

TMIC-36. LOCAL TISSUE BIOMARKERS OF RESPONSE TO THERAPY FOR GLIOBLASTOMA

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Glioblastoma (GBM) is a common deadly malignant brain cancer of the central nervous system (CNS), with a median survival of 12–15 months. Scientific advancements are lacking in developing effective therapies for both primary GBM, as well as secondary GBMs, that typically originate as malignant transformation of lower-grade isocitrate dehydrogenase (IDH)-mutant tumors. The unique metabolomic profile of IDH-mutant tumors may present opportunities to develop biomarker signatures of therapeutic efficacy. Microdialysis is an extracellular fluid sampling collection technique utilizing a perfused semipermeable catheter to permit diffusion of molecules between brain interstitium and the perfusate. We hypothesized that microdialysis may identify a metabolomics-based biomarker response to therapy in IDH-mutant tumors. To test this hypothesis, orthotopic xenografts were generated from two patient-derived GBM lines harboring mutations in IDH1. Perfusates were collected from intra-cranial tumors in aythmic nude mice sampled at baseline, 24h and 72h time-points post treatment with temozolomide, an oral alkylating agent used to treat IDH-mutant gliomas, compared with vehicle treatment, and TMZ-treated non-tumor bearing animals. Perfusates were analyzed via unsupervised metabolomic profiling using both gas and liquid chromatography-mass spectrometry (GC/LC-MS). Results of metabolic signatures will be presented. This study aims to demonstrate proof-of-principle and the feasibility of using microdialysis as an approach to identify local tissue biomarkers of tumor response to drug therapy. This work will be complemented by parallel analysis of non-IDH-mutant and TMZ-resistant xenografts, as well as signatures of response to other therapies to yield predictive *in vivo* tissue biomarkers of drug responsiveness translatable to clinical practice.

TMIC-37. TARGETING THE EXTRACELLULAR MATRIX OF GLIOBLASTOMAS CHANGES THE POLARIZATION OF TUMOR-ASSOCIATED MACROPHAGES AND INCREASES ANTI-TUMOR INFLAMMATORY RESPONSES

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Glioblastomas (GBMs) are heavily infiltrated by tumor-associated microglia and macrophages (TAMs), which represent the predominant immune cell population in the tumor and may contribute to GBM progression in response to signals from the tumor cells. Fibulin-3 is an extracellular matrix protein secreted by GBM cells that promotes tumor invasion, angiogenesis, and survival of the GBM stem cell population (GSC) by activation of ADAM17/NFkB signaling. We recently developed a function-blocking antibody ("mAb428.2") against fibulin-3 that inhibits the molecular mechanisms of this protein and reduces GSC viability (Nandhu et al., *Clin. Cancer Res.* 2018). mAb428.2 (8 days x 30 mg/kg, q24h, IV) exerted a significant anti-tumor effect and prolonged the survival of mice carrying patient-derived GBM xenografts implanted sub-cutaneously (N=8/arm p=0.0032) or intracranially (N=10/arm p=0.0005). Importantly, the cyto-reductive effect of mAb428.2 was accompanied by significant necrosis of the tumor core and increased infiltration of IBA1-positive TAMs. Remarkably, TAMs in anti-fibulin-3-treated tumors showed very little expression of Arginase-1, a bona-fide marker of M2 ("tumor-promoting") polarization. Analysis of gene expression in the TAMs recovered from the tumors confirmed significantly increased expression of inflammatory cytokines (IFN-gamma, IL-1beta, IL-10) and downregulation of M2 markers (CD163, CD206, ARG1), suggesting that mAb428.2 had blocked the usual M2 polarization of TAMs observed in GBMs. Moreover, mAb428.2-treated tumors also showed drastically decreased expression of CSF-1a, an M2-inducing cytokine. Knockdown of fibulin-3 in GSCs also reduced CSF-1a expression, which is likely regulated by fibulin-3 through NFkB signaling. Finally, co-culture of PMA-activated U937 macrophages with GSCs caused a significant decrease of tumor cell viability when mAb428.2 was added to the cultures, suggesting that anti-fibulin-3 treatment enables TAMs to attack tumor cells. Taken together, our results strongly suggest that anti-fibulin-3 and possibly other anti-ECM approaches may induce a marked inflammatory reaction driven by innate immune cells, potentiating anti-tumor therapy.

TMIC-38. ENHANCED EFFICACY OF TUMOR TREATING FIELDS AND AURORA B KINASE INHIBITOR COMBINATION IN GLIOMA CELL LINES

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Tumor Treating Fields (TTFields) are anti-mitotic, low intensity, intermediate frequency, alternating electric fields approved for glioblastoma. The efficiency of TTFields may be enhanced by drugs that extend metaphase-anaphase transition and telophase such as Aurora B kinase. We studied if TTFields' effect on tumor cells is enhanced by additional inhibition of cytokinesis through Aurora B kinase. TTFields and Aurora B kinase inhibitors (AZD1152) combination was tested in glioma cell lines: in U87-MG, U87-MG^{shP53} and U-251, and U87-MG, first and then in two primary glioblastoma cell lines (HT16360, HT18503). TTFields (1.6 V/cm RMS, 200 kHz) were applied for 72 hours using the inovitro system. AZD1152 was added up to 100 nmol/L. Cell counts, cell cycle and clonogenic potential were determined. Multinuclear cells formation was determined using crystal violet staining. TTFields and AZD1152 combination led to a significant reduction in the number of U251, U-87 MG and U-87 MG^{shP53} cells (2-way ANOVA, pshP53 and U-251 cells (2-way ANOVA, pshP53 cells stained with crystal violet after treatment revealed high prevalence of multi nuclear cells in cells exposed to TTFields and AZD1152 (25nM) versus AZD1152 (25nM) alone. Cells treated with TTFields and higher doses of AZD1152 (50-100nM) demonstrated increased rates of pyknosis. Data could be confirmed in primary tumor-cell lines. These results demonstrate that combining TTFields and AZD1152 can be an effective treatment in glioma. There is a strong rational to continue exploring the potential of combining TTFields and AZD1152 in the clinical settings.

TMIC-39. UNRAVELLING THE TUMOUR MICROENVIRONMENT OF GLIOMA

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BACKGROUND: With the incidence and mortality of brain cancer almost equivocal there is a vital need to understand the intricacies of the glioma microenvironment. This study details the temporal and spatial localisation of immune cell populations and mediators during glioma development using the murine orthotopic Gl261 glioma model. METHODS: Male C57Bl/6 mice were stereo-tactically inoculated with $1x10^6$ Gl261 cells at AP0.1mm, ML1.0mm, DV2.4mm Bregma. Immune cell populations were assessed at D0, D1, D3, D7, D14, D21 post-inoculation by veterinary haematological analyser (Coulter Ac·Tdiff™) and 16-parameter flow cytometry (Fortessa™). RESULTS: Significant temporal changes were observed in all immune cell populations among the splenic, bone marrow and peripheral blood systemic compartments: WBCs, RBCs, platelets, $CD3^+$ T

cell, $CD3^+CD4^+$ Th, $CD3^+CD4^+CD25^+$ Treg, $CD3^+CD8^+$ Tc, $NK1.1^+$ NK, $NK1.1^+CD3^+$ NK/T, $CD115^+CD11b^+$ monocyte, $CD115^+CD11b^+CD80^+$ M1, $CD115^+CD11b^+CD206^+$ M2, $CD115^-CD11b^+$ DC, $CD115^-CD11b^+Ly6C^{high}Ly6G^-$ M-MDSC, $CD115^-CD11b^+Ly6C^+Ly6G^+$ PMN-MDSC, $CD117^+$ HSC and $CD19^+$ B cell (Kruskal-Wallis with Dunn's multiple comparison test; p=0.02- p< 0.0001). Analysis of plasma coagulation and inflammatory mediators, and histopathology of the tumour microenvironment is to be completed. CONCLUSIONS: The data derived provides baseline characteristics to study the changes associated with radiation, chemotherapy and immunotherapy treatment and to sequence therapies to maintain immune modulation. This information will contribute to identifying those immunotherapies which will have maximal benefit among the glioma patient population, where currently anti-CTLA-4 and anti-PD-1 immunotherapies have had little impact. KM is supported by a Matt Callander Beanie for Brain Cancer HMRI Fellowship funded by the Mark Hughes Foundation, and grants from The Brain Cancer Group and Sydney Vital Translational Cancer Centre; AH is supported by a The Brain Cancer Group Fellowship.

TMIC-40. INTERLEUKIN-6 IN CEREBROSPINAL FLUID AS A PROGNOSTIC MARKER FOR GLIOBLASTOMA PATIENTS

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INTRODUCTION: Inflammatory cells are key components of the tumor microenvironment, and cytokines could create a favorable microenvironment to support tumorigenesis. Several studies have reported that IL-6 levels in glioma tissues are associated with the prognosis of patients with glioma; however, IL-6 levels in cerebrospinal fluid (CSF) of glioma patients has not been sufficiently studied yet. METHODS: The patients with gliomas were treated at the Department of Neurosurgery, University of Kobe, between January 2007 and December 2016. CSF was withdrawn from the patients via lumbar puncture, and IL-6 concentration was measured by ELISA. after obtaining informed consent when not contraindicated. Infiltration of tumor-associated macrophages (TAMs) were analyzed by CD163 staining. RESULTS: Seventy-six patients with gliomas were analyzed in this study. The median follow-up period was 25 months. At the end of the follow-up period, 22 patients were alive, and 53 patients were dead. In the pretreatment CSF examination, mean concentration of IL-6 was 26.3 ± 88.5 pg/ml. In glioblastoma alone, the mean value of CSF IL-6 was 35.6 ± 102.5 pg/ml, which was significantly higher than that of other grades of gliomas. The concentration of CSF IL-6 was correlated with the concentration of CSF LDH. IL-6 protein was expressed in the area where TAMs were predominantly existed. In double immunostaining examination, IL-6 protein was expressed in TAMs, and the concentration of CSF IL-6 was statistically correlated with the percentage of TAMs infiltration in the tumor (p=0.014). In multivariate analysis, high levels of CSF IL-6 were significantly associated with shorter overall survival (HR=1.007, 95% CI: 1.002~.012, p=0.0049) CONCLUSIONS: These results indicated that the concentration of CSF IL-6 may reflect the microenvironment of the tumor and may be a useful prognostic biomarker for glioma patients.

TMIC-41. APOPTOTIC CELLS PROMOTE MALIGNANCY OF SURVIVING GLIOBLASTOMA CELLS BY INTERCELLULAR TRANSFER OF SPLICING FACTORS

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Because of the pressure of rapid expansion of tumor, intratumoral microenvironment becomes inevitably harsh, resulting in abundant apoptotic tumor cells intermingled with neighboring proliferating cells. Recent studies have suggested that signals from dying cells may play an important role in the progression of various types of malignancies including glioblastoma (GBM). Nonetheless, mechanisms involved in the transduction of signal between apoptotic and surviving tumor cells remains elusive. Our findings recently published in Cancer Cell journal revealed that glioblastoma cells can specifically secrete RNA and protein components of spliceosomes after the courses of chemo- and radiotherapy. Further experiments demonstrated that spliceosomal proteins are exported by apoptotic cancer cells within exosome-like extracellular vesicles (EVs). These EVs induce pre-mRNA splicing changes in recipient glioblastoma cells and promote proliferation, migration, therapy resistance and aggressive mesenchymal phenotype of glioblastoma patient-derived neurospheres both in vitro and in vivo. Mechanistically, we identified RBM11 as a representative splicing factor that is upregulated in tumors after therapy and shed in EVs upon induction of apoptosis. Once internalized in recipient cells, exogenous RBM11 switch splicing of MDM4 and CyclinD1 towards the expression of more oncogenic isoforms which is associated with worse patient prognosis. Taken together, our findings reveal a novel role of spliceosomal proteins in intercellular communication between apoptotic and surviving tumor cells. It is likely that this mechanism is applicable to cancer types beyond GBM. Clinically, our data may provide the rationale for the molecular targeting of RNA splicing events or specific splicing factors to attenuate the gain of post-therapeutic malignant changes in GBM.

TMIC-42. DEVELOPMENT OF NOVEL SPRAY-TYPE FLUORESCENT PROBES FOR BRAIN TUMORS

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PURPOSE: 5-Amino Levulinic Acid (5-ALA) is commonly used as an intraoperative aid in malignant glioma surgery, which has been proved to be effective for more radical tumor resection and better patient prognosis. However, there are some limitations in its use, such as false positivity, false negativity, and inability of re-administration. We aim to develop a novel fluorescent labeling system, which can be repeatedly administered by spray during surgery, using hydroxymethyl rhodamine green (HMRG) and 2 methyl siliconrhodamine (2MeSiR600) as host fluorescent nuclei originally designed at our university for cancer detection, complementing 5-ALA. METHODS: Four groups of homogenized samples were prepared from frozen tissues which consisted of 10 peritumoral specimens, 5 glioblastomas, 5 astrocytomas, and 5 oligodendrogliomas. Probe screening was performed using the fluorescent probe library comprised of HMRG and 2MeSiR600 host fluorescent nuclei combined with various types of dipeptides. More than 720 kinds of fluorescent probes were applied to homogenized lysates. According to the fluorescence intensity measured over time after application, probes exhibiting strong focal fluorescent marks with large difference between peritumor and tumor tissues were selected as valid probes. The selected probes were then validated by the experiment using fresh specimens. RESULTS & DISCUSSION: The top probes were selected based upon the experiments using homogenized lysates as well as fresh specimens. They were validated prospectively with more surgical cases. With the combination of the two types of fluorescent host nuclei, glioblastoma can be identified in multi-color. CONCLUSION: Fluorescent probes with HMRG and 2MeSiR 600 host nuclei can be effective for intraoperative detection of glioma.

TMIC-43. A TENSION-MEDIATED GLYCOCALYX FEEDBACK LOOP PROMOTES A MESENCHYMAL, STEM-LIKE PHENOTYPE IN GLIOBLASTOMA

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Glioblastoma multiforme (GBM) is one of the deadliest central nervous system cancers, difficult to treat and largely impossible to cure. A considerable challenge in GBM therapy is treatment resistance and tumor recurrence, resulting in dismal patient prognosis. GBM aggression is often associated with a mesenchymal phenotype, increased expression of glycoproteins and the presence of tumor-initiating "stem-like" cells. Using patient-derived xenograft models, and immune competent syngeneic and transgenic glioma mouse models, we show that GBMs demonstrate increased contractility, and their tumors are surrounded by a stiffer ECM, that maintains and even further enhances integrin mechanosignaling. As a result of increased integrin signaling, these tumors also harbor a mesenchymal-like gene signature and

phenotype, possess a bulky glycocalyx and demonstrate a stem-like phenotype. Bioinformatics analysis, expression profiling and limiting dilution studies reveal that these mechanically challenged tumors express more "bulky" glycoproteins such as mucins and CD44, as well as glycocalyx regulators, galectins. Considering that a large proportion of these bulky glycoproteins are also stem markers, we show that upregulation of the glycoproteins and their modulators leads to enhanced GBM stem-ness. Our findings suggest that there is a dynamic and reciprocal link between integrin mechanosignaling and a bulky glycocalyx, which promotes a mesenchymal, stem-like phenotype in GBMs. Thus, therapeutic strategies to target GBM tissue tension could reduce mortality and improve patient outcome.

TMIC-44. ASTROCYTE SENESCENCE: A MODEL FOR AGE AND SEX EFFECTS ON GLIOBLASTOMA INCIDENCE

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Primary brain tumor incidence rates rise more rapidly in males than females with increasing age. Both pre-neoplastic cells and stromal elements contribute to age-related increases in cancer, and cellular senescence plays a role in the transformation of pre-neoplastic cells. While senescence prevents the proliferation of damaged cells, senescent cells produce senescence-associated secretory phenotypes (SASP) which contribute to the tumor microenvironment. Certain SASP factors favor tumor suppression by inducing senescence in neighboring cells or recruiting immune cells for tumor clearance, while other factors increase tumor proliferation, resistance and relapse. Studies have demonstrated the role of senescence in the growth of solid tumors in various tissues including the liver, pancreas, and prostate. However, no studies have investigated how senescence in resident brain cells influences age-dependent increases in brain tumors. Moreover, there are no data in any tissues about sex differences in cellular senescence and its effect on age-dependent cancer rates. In the brain, astrocytes are the most abundant resident cell type with critical functions in brain structure, physiology, and homeostasis. Thus, we investigated sex differences in astrocyte senescence and whether these differences contribute to the sex disparity in brain tumor rates, specifically glioblastoma which has a male to female ratio of 1.6. Using β-galactosidase staining, we found that murine female astrocytes undergo senescence more readily after oxidative stress than their male counterparts. Furthermore, *in vitro* studies showed that murine GBM cells cultured in female astrocyte SASP-conditioned media show a different pattern of growth as compared to those cultured in male astrocyte SASP-conditioned media. These results indicate that there are sex differences in astrocyte senescence that influence tumor proliferation. Further investigation into the molecular mechanisms involved in male and female senescent astrocytes will allow us to better understand age-dependent brain tumor growth, which may reveal novel therapeutic targets for treatment.

TMIC-45. OLIGODENDROCYTE PROGENITOR CELLS AND MACROPHAGES/MICROGLIA INDUCE CHEMO-RADIORESISTANT ABILITIES IN GLIOMA CELLS AT THE TUMOR BORDER

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Glioblastoma (GBM) usually develops in adult brain white matter and shows rapid growth and invasion. Even after complete resection, GBM recurs around the tumor removal cavity, where GBM cells acquire chemo-radioresistance and survive. Characterization of the tumor border microenvironment is critical for improving prognosis in patients with GBM. Here, we compared microRNA (miRNA) expression in samples from the tumor, tumor border, and peripheral region far from tumor mass by miRNA microarray. The top three miRNAs showing higher expression in the tumor border were related to oligodendrocyte differentiation, and pathological oligodendrocyte lineage cells increased in the border, where numbers of macrophages and microglia also colocalized. Medium cultured with oligodendrocyte progenitor cells (OPCs) and macrophages induced stemness and chemo-radioresistance in GBM cells, similar to that produced by FGF1, EGF and HB-EGF, IL-1β, corresponding to OPCs and macrophages, respectively. Thus, OPCs and macrophages/microglia may form a glioma stem cell niche at the tumor border, representing a novel, promising target for prevention of recurrence.

TMIC-46. FIBROBLAST-PRODUCED EDA FIBRONECTIN IN THE SUBVENTRICULAR ZONE DRIVES GLIOBLASTOMA PATHOGENESIS

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INTRODUCTION: Glioblastoma is an aggressive cancer with a dismal median survival of under two years. Recent studies have shifted the paradigm of glioblastoma from a homogenous mass of tumor cells into a complex organ containing interacting elements. We sought to determine whether cancer-associated fibroblasts (CAFs) are among these elements in glioblastoma. METHODS: We collected site-directed biopsies from glioblastoma patients. Ex vivo analysis was performed using immunohistochemistry (IHC), quantitative reverse-transcription PCR (RT-qPCR), fluorescence-activated cell sorting (FACS), Matrigel invasion and CyQuant proliferation assays. RESULTS: FACS of site-directed biopsies determined that PDGF receptor alpha+ CAFs comprised 9% of cells in the glioblastoma subventricular zone (SVZ), a region known to harbor glioblastomas with a worse prognosis, vs. 2% of cells outside the SVZ in glioblastomas and were not present in SVZ specimens from epilepsy cases (p=0.02). CAFs were chemotactically attracted to the glioblastoma stem cells found in the SVZ. In vitro culture of EDA-FN, a molecule secreted by CAFs, with macrophages resulted in a 16-fold increase in pro-tumoral genes when compared to non-EDA expressing FN (p=0.0001). The expression level of EDA-FN was directly and significantly associated with upregulated mesenchymal genetic markers (p<0.001), which have also been associated with worse patient outcomes. IHC revealed that EDA-FN forms a scaffolding network and is spatially associated with CAFs. CONCLUSION: We defined a cascade by which tumor stem cells in the SVZ recruit CAFs, which in turn produce EDA-FN in the SVZ. EDA-FN induces M2 polarization and is associated with mesenchymal gene expression. Given the specificity of CAFs and EDA-FN to cancer, both represent viable therapeutic targets.

TMIC-47. GLIOMA STEM CELL-VASCULAR INTERFACE IN GLIOBLASTOMA PROGRESSION

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Glioblastoma (GBM) is a highly vascularized, invasive and therapy-resistant brain tumor driven by glioma stem-like cells (GSCs). GSCs reside in three specialized niches or microenvironments: the perivascular, hypoxic and invasive niches. The perivascular and hypoxic niches support the maintenance of GSCs within the tumor bulk, whereas the invasive niche consists of GSCs associated with the pre-existing blood vessels that they co-opt to migrate and invade into other brain regions. Recent findings indicate a bidirectional conversion between the perivascular maintenance and invasive niche propelled by the enormous plasticity of GSCs to transdifferentiate into endothelial-like cells (ECs) and pericytes (PCs). Importantly, these GSC-derived EC-like cells are predominantly found in recurrent tumors than matched primary tumors indicating that therapy may promote GSC plasticity and transdifferentiation into vascular elements. The standard treatment for most GBM patients is maximal surgical resection, fractionated radiation and adjuvant chemotherapy with the drug temozolomide. Prior studies demonstrated that radiation therapy (RT) can induce reprogramming and de-differentiation of tumor cells into a GSC-like state. Based on these findings, we tested the effects of RT in promoting the transdifferentiation potential of GSCs into vascular elements. Our *in vitro* findings show that RT induces EC gene signature and promotes angiogenic activity in GSCs. We also see that radiated GSCs from tumor core and margins embedded in 3D-hydrogel system with varying stiffness that mimics the tumor ECM and microenvironment exhibit differential capacity to form the vascular elements. Xenografted GBM tumors exposed to RT showed enhanced GSC conversion to vascular cells indicating that RT indeed promotes GSC transdifferentiation into vascular elements *in vivo*. Ongoing experiments are aimed at determining the molecular mechanism, and functional consequences of ablating the RT-induced GSC-vascular interface on tumor growth and recurrence.

TMIC-48. DYNAMIC OF MICROGLIA POLARIZATION IN GLIOMA TUMOR RESECTION AREA

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All glioma tumors relapse after the surgical resection. Microglia is an important component of the tumor microenvironment and provides support for tumor growth. Both M1 pro-inflammatory and M2 pro-tumorigenic microglia were found in gliomas with predominance of M2. Surgical resection of brain tumors results in local tissue damage, causing microglial activation in the surgical area. We hypothesize that tumor resection provides signaling for microglial polarization modifications and exacerbates the ma-

lignant properties of the tumor cells that fail to be eliminated by resection, stimulating the tumor recurrence. The purpose of this study was to evaluate the dynamics of microglial polarization in the tumor resection area during the post-surgical period. C57BL/6/GL261 mouse glioma implantation model was used. M1 and M2 microglia were identified in area of tumor resection using western blot and immunofluorescent approaches (CD86, iNOS, CD206 and Arginase 1 were used as markers of M1 and M2 microglia). Study has been performed 0, 2 hours, 2, 6, and 10 days after the tumor resection. The dynamic shift in the immunological status of microglia have been found during the healing of the resected area. Up regulation of M2 microglia has been detected beginning 2 hours after tumor resection with further reduction by day 10. Same but less prominent dynamic of M1 microglia has been revealed. Electron microscopy demonstrated accumulation of phagocytic microglia in the immediate area of the resection edge with non-phagocytic microglia accumulated deeper in the tissue. These results suggest that the tumor resection procedure stimulates the polarization of both pro-tumorigenic M2 and inflammatory M1 microglia up to 10 days after tumor resection with the predominant accumulation of M1 microglia at the resection rim and accumulation of M2 deeper in the parenchyma. Acknowledgement: NIH Grants 1SC1GM122691 and R25GM110513.

TMIC-49. ACTIVATION OF THE Wnt/β-CATENIN SIGNALING PATHWAY IN GLIOMA STEM CELLS IMPACTS ENDOTHELIAL CELL-CELL INTERACTION

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BACKGROUND: The presence of invasive glioma stem cells (GSC) in glioblastoma (GBM) enhances therapy resistance and disease recurrence. Low permeability and high selectivity of the blood-brain barrier (BBB) limits CNS drug delivery of effective treatment against GSC growth. Moreover, increased Wnt/β-catenin signaling in GBM is associated with radio-resistance and proliferation. Interestingly, the Wnt-subtype of medulloblastoma is characterized by activating mutations of β-catenin and associated with a good prognosis linked to a highly permeable BBB. We propose that activation of WNT/β-catenin signaling in GSCs will decrease brain endothelial cell-cell junctional integrity thereby increasing BBB permeability in the tumor microenvironment. METHODS: We used CHIR99021, a glycogen synthase kinase 3β inhibitor, to activate Wnt/β-catenin signaling in a human derived primary GSC line, GSC923. Human brain microvascular endothelial cells (HBMEC) were treated with conditioned media from GSC923 treated with or without CHIR99021 for 24 hours. Endothelial barrier function was evaluated by electrical cell impedance using the ACEA xCELLigence system. Data was reported by changes in cell index to reflect cell-cell interaction integrity. Junctional expression was evaluated by qPCR, immunoblotting and immunofluorescence. Mass spectrometry of GSC media was evaluated for specific Wnt/β-catenin downstream proteins which impact the BBB. RESULTS: HBMEC cell index was decreased by approximately 60% when treated with conditioned medium from Wnt/β-catenin activated GSC923 cells; suggesting decreased cell-cell interactions and increased permeability. Gene expression of the endothelial junctional proteins, CLAUDIN-3, -5, OCCLUDIN, ZO-1, VE-CADHERIN, PECAM1, and CTNNB1 were decreased by approximately 20–50%. Immunofluorescence and mass spectrometry analysis studies are ongoing. CONCLUSION: Our findings suggest that disruption of brain endothelial junctional interactions occur in a paracrine manner under the Wnt/β-catenin signaling axis from glioma stem cells to HBMEC. Modulation of intratumoral Wnt/β-catenin signaling, particularly in highly resistant GSCs, may enhance chemotherapy drug delivery, potentially expanding the drug portfolio and improving the prognosis of GBM.

TMIC-50. HYPERACTIVATING THE HIPPO PATHWAY EFFECTOR TAZ DISTORTS THE TUMOR MICROENVIRONMENT, PROMOTES TUMOR-ENTRAINED NEUTROPHIL INFILTRATION, AND PHENOCOPIES MESENCHYMAL-GLIOBLASTOMA

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Glioblastoma (GBM), the deadliest and most common primary CNS malignancy in adults, is highly heterogeneous with variable prognoses and treatment responses. The molecular underpinnings for such variability remain largely unclear. Mesenchymal (MES)-GBMs, associated with poorest prognosis and highest treatment resistance, exhibit hyperactivity of *transcriptional coactivator with PDZ-binding motif* (*TAZ*), the major effector of the Hippo tumor suppressive pathway. Additionally, the tumor microenvironment (TME) of MES-GBMs appears to be altered, containing more infiltrative tumor-associated macrophages and microglia than GBMs of other subtypes. Moreover, the Hippo pathway has recently been shown to be involved in suppressing tumor immunogenicity. Yet, it remains elusive whether TAZ dysregulation distorts the TME to contribute to MES transition and tumor progression of MES-GBMs. We suspected that such TME distortion facilitates tumor immune evasion, MES transition, and tumor progression, therefore worsening prognoses and treatment responses. To test this, we generated three GBM cell lines expressing various forms of TAZ: 1) GBM^{4SA} expressing constitutively-active TAZ 2) $GBM^{4SA\text{-}S51A}$ expressing TAZ incapable of binding to its downstream effector, TEAD, and 3) GBM^{vector}, vector control. We found mice orthotopically implanted with GBM^{4SA} phenocopied MES-GBM patients and exhibited significantly shorter survival. Histopathology revealed that GBM^{4SA} highly resembles human GBMs, with high cellular and nuclear anaplasia, microvascular proliferation, and pseudopalisading necrosis. Moreover, TME analyses from these GBMs suggested that GBM^{4SA} contains significantly more infiltrative myeloid cells than $GBM^{4SA\text{-}S51A}$ or GBM^{vector}, suggesting that hyperactivity of TAZ leads to an altered TME. While most infiltrative myeloid cells in the GBM^{vector} or $GBM^{4SA\text{-}S51A}$ TME were of monocyte lineage, those in the GBM^{4SA} TME were predominantly granulocytic, likely representing neutrophils. We will further delineate the roles of each myeloid cell type in the MES-GBM TME in driving tumor progression and treatment resistance, and we hope to draw implications for future GBM clinical management and novel TME-targeted anti-GBM therapies.

TMIC-51. DEXAMETHASONE DRIVES MYELOID-DERIVED SUPPRESSOR CELL ACCUMULATION IN GLIOBLASTOMA IN A SEX-SPECIFIC MANNER

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Myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of immunosuppressive cells consisting of monocytic (M-MDSC) and granulocytic (G-MDSC) subsets that accumulate in tumors, where they constrain anti-tumor immunity. High MDSC frequency in tumor and blood correlates with poor glioblastoma (GBM) prognosis and limits patient response to conventional and immunotherapy treatment strategies. Therefore, understanding factors regulating the dynamic behavior of MDSCs can improve the treatment of GBM. We examined the impact of sex as a biological variable and dexamethasone (DEX) use as a standard disease management strategy on glioma-associated MDSCs activity. Our results showed that M-MDSC and G-MDSC had unique gene signatures and were differentially recruited to mouse GBM tumors. Despite increased G-MDSC production in the bone marrow of tumor-bearing mice, M-MDSCs infiltrated tumors at a higher rate. This frequency was especially higher in male mice, which reached morbidity endpoint earlier than female mice. Under *in vitro* culture conditions favoring myeloid cell polarization, DEX improved the generation MDSCs from bone marrow precursors by >4-fold; and increased the frequency of resting M0 and immunosuppressive M2 macrophages by 2-fold. DEX also blocked MHC Class II expression in pro-inflammatory M1 macrophages, impairing antigen presentation. These results were validated in a preclinical model of GBM. Daily DEX administration to tumor-bearing mice accelerated the onset of disease-related neurological symptoms and elevated the frequency of M-MDSCs in tumors of male mice. This change in the tumor immune profile was accompanied by reduction of pro-inflammatory mediators IL-12p70, TNFa and IP-10 in plasma, and increased levels of G-CSF, a strong stimulator of MDSCs. Our findings suggest that standard disease management with DEX may augment the immunosuppressive tumor microenvironment, which could be reversed by targeting MDSCs. Sex-related immunological differences can also enable the development of personalized medicine approaches that seek to effectively activate the immune system to attenuate tumor growth.

TMIC-52. DEXAMETHASONE-MEDIATED ACTIVATION OF FIBRONECTIN MATRIX ASSEMBLY INHIBITS DISPERSAL OF HUMAN PRIMARY GBM CELLS IN A MOUSE RETINA IN VIVO MODEL

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INTRODUCTION: Identifying drugs that can mitigate dispersal, particularly after patients undergo the Stupp protocol, may increase the length of time to radiological recurrence and improve overall survival. Previous studies have shown that Dexamethasone (Dex) treatment induces fibronectin matrix assembly (FNMA) and that this significantly reduces dispersal of primary human GBM (pGBM) cells in vitro and ex vivo. Here, we introduce an in vivo mouse retina dispersal assay to demonstrate that Dex also inhibits dispersal in vivo. METHODS: Single cell dispersions of fluorescently-labeled pGBM cells were injected into mouse retinas along with soluble fibronectin, and mice were either treated with vehicle control or Dex at dose equivalents of 1mg-

8mg/day for 5 days, whereupon mice were sacrificed and eyes removed and fixed in 4% PFA. Retinas were then extirpated, stained with Fn antibody and DAPI, mounted, and imaged by scanning laser confocal microscopy. z-stacks were collected and penetration distance assessed. 3-color images were generated for localization of pGBM cells, FNMA and DAPI. Additionally experiments were performed in the presence of Dex and either FUD fragment, an inhibitor of FNMA, or iii-11C control peptide. RESULTS: We show that Dex significantly reduces z-axis penetration of pGBM cells into mouse retina, that Dex treatment significantly alters the morphology of dispersal of injected pGBM cells within the x,y plane, that without Dex, the presence of fibronectin increases dispersal, that Dex treatment activates FNMA by pGBM cells leading to containment of the tumor mass, and that Dex-mediated activation of FNMA is fibronectin dose-dependent. Moreover, treatment with FUD but not iii-11C restores ability of tumor cells to disperse. Interestingly, dispersal inhibition could be achieved at Dex doses as low as 1mg/day. CONCLUSION: Our study defines a role for fibronectin as a facilitator of pGBM dispersal and Dex-mediated activation of FNMA as an inhibitor of that process.

TMIC-53. IDENTIFICATION OF MYELOID CELL-DERIVED TRANSCRIPTS IN GLIOBLASTOMA

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Glioblastoma-associated microglia and macrophages/monocytes (GAMs) constitute the most abundant type of immune cells in glioblastoma. To date there is still insufficient knowledge about the expression profiles and activation states of these cells. We have previously performed RNA-Seq and microarray on CD11b+ freshly isolated human and mouse GAMs. Here we combine this data with analysis from published whole tumor TCGA data to identify new myeloid-derived transcripts in glioblastoma. We first re-analyzed our previously published human GAMs RNA-Seq dataset for genes that are at least 4-fold up regulated in comparison to naïve microglia and displayed an absolute log2 expression greater than 7. Subsequently, we queried the TCGA glioblastoma dataset to identify genes that showed a high correlation to the myeloid markers *CD68*, *CD14*, and *AIF1*. We found that the expression of *IBSP*, *FCGBP*, *MARCO*, *RNASE1*, *CTSL*, *SPP1*, and *GPNMB* significantly correlated with the expression of the different myeloid markers. In addition, the correlation was the highest in the proneural subtype samples, which have previously been shown to harbor the highest percentage of myeloid cells. Three of these genes (*Spp1*, *Ctsl*, and *Gpnmb*) were also highly expressed in murine GAMs. Finally, we performed immunofluorescence stainings for CTSL and GPNMB in human GBM samples and PDGF-driven mouse tumors and could show that both proteins were highly abundant in IBA1+ myeloid cells in both human and mouse samples. Conclusively, we have identified several new myeloid-specific genes in glioblastoma that might pose potential new targets. Subsequent studies will be necessary to evaluate the role of these genes in glioblastoma.

TMIC-54. THE IMPACT OF GLIOMA CANCER CELL STEMNESS ON EXOSOME PHENOTYPE

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Glioblastoma is the most common central nervous system malignancy in adults with a median survival of only 14 months. The current arsenal of treatment modalities including surgical resection and combination chemoradiation have been largely ineffective. One reason proposed for the ineffectiveness of our current therapeutic regimen is the resistance of glioma stem cells (GSCs) within the tumor and tumor borders. We investigated the hypothesis that the communication of GSCs to their microenvironment through exosomes is a key factor underlying the enhanced cellular proliferation and the development of resistance to therapeutics. Exosomes are nanometer sized vesicles released by cancer cells that contain DNA, RNA, and protein critical to the interaction of a cell with its microenvironment. Two properties of exosomes were analyzed: 1) exosome function and 2) exosome profile. Exosomes secreted by patient derived-glioma stem cells (GSC-exosomes) increased cellular proliferation, radiation resistance, temozolomide resistance, and doxorubicin resistance. We further profiled the GSC-exosomes to begin to probe the underlying mechanism of this phenomenon. Profiling showed specific changes to RNA and protein favoring therapeutic resistance and cellular proliferation. For example, GSC exosomes have increased expression of proteins involved in radiation and chemotherapeutic resistance (Ex. CDK4 and Notch), cellular proliferation (Ex. Cyclin B1 and Cyclin D2), angiogenesis (Ex. VEGF-A and EGFR), glioma cell stemness and de-differentiation (Ex. EPHA2, Cathepsin B), and cell invasion and metastasis (Ex. ITGA3, COL4A2). The results of our study suggest a novel exosome-based mechanism by which glioma stem cells alter therapeutic resistance and increase cellular proliferation.

TUMOR MODELS

TMOD-01. THE ROLE OF PERSISTENCE, PROLIFERATION, AND TUMOR CELL KILLING EFFICIENCY IN DETERMINING RESPONSE TO CAR T-CELL THERAPY IN GLIOBLASTOMA: A MATHEMATICAL MODEL AND ANALYSIS

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Chimeric antigen receptor (CAR) T-cell therapy is a promising emerging area of immunotherapy for the treatment of recurrent glioblastoma. In this therapy, T-cells are genetically modified to target tumor-specific antigens. Efficacy of CAR T-cell therapy depends on several factors, including CAR T-cell proliferation, persistence, and tumor cell killing capacity. Here we use a mathematical model to investigate the role of these three factors in determining response of recurrent GBM to this novel immunotherapy. Additionally, the impact of dosing and scheduling of CAR T-cell infusions on the therapeutic response is explored. We model the interaction between the two cell populations (cancer cells and CAR T-cells) using an ordinary differential equation based formalism. The growth and death of cancer cells are simulated as rates of proliferation and interaction between cancer cells and CAR T-cells, respectively. Biological data was used to parameterize the model. Analysis of the dynamics of interaction between cancer cells and CAR T-cells was performed to determine the maximum efficacy of a single and multiple doses of CAR T-cells. Our mathematical model and analysis shows that a critical parameter for the success of CAR T-cell therapy is the ratio of cancer cell proliferation to the killing capacity of the CAR T-cells. We quantify the dose level of CAR T-cells required to eliminate the cancer cell population. Furthermore, we use the mathematical model to predict the time to progression for specific dose levels, which may help in optimizing and scheduling multiple doses of CAR T-cell monotherapy. We compare the mathematical results with patient data from an ongoing dose-escalation study (NCT02208362) using central memory derived IL13Rα2-targeted CAR T-cell therapy for recurrent glioblastoma.

TMOD-02. DIFFUSE GLIOMATOSIS IN MOUSE MODEL OF GFAP TISSUE SPECIFIC KNOCK IN OF EGFRvIII AND KNOCK OUT OF p19 ARF

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We generated a transgenic mouse harboring EGFRvIII knock in and homozygous p19 ARF deletion restricted to glial cells. The mice developed diffuse glioma with some tumors showing classical histological pseudopalisading necrosis and hyper vascularity. Transgenic mice develop tumors as early as 1 month and 53% of mice die by 6 months of age. MRIs reveal that mice develop ventriculomegaly and external hydrocephalus when tumor cell infiltration grows exophytic and presents with accumulations of neoplastic cells in association with leptomeninges, in addition to the brain and spinal cord parenchyma. Immunohistochemical analysis shows a strong glial component; tumors display positive GFAP, S100 staining and negative NeuN, synaptophysin staining. Three primary cell cultures were isolated as adherent and neurospheres from mice with tumors. During in vitro culture, EGFRvIII expression was lost in 2 of 3 lines, similar to seen in humans GBMs. Results were confirmed by Western blot and RT-PCR. Intracranial injection with EGFRvIII positive lines resulted in tumors in NODSCID mice. Immunohistochemistry staining of xenografts show that cells retained EGFRvIII expression, thus reinforcing that the importance of tissue microenvironment in maintaining genomic drivers. We present phenotype and genomic profile of a new transgenic model of glioma, with a prominent phenotype of leptomeningeal spread, potentially serving as a model for this important manifestation of human tumors.

TMOD-03. GLIOMAPDOX: A MOLECULARLY DIVERSE LIBRARY OF DIRECT-FROM-PATIENT ORTHOTOPIC GLIOMA XENOGRAFTS RECAPITULATES INTRATUMOR HETEROGENEITY

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The development of effective treatments for malignant brain tumors has been hampered by the lack of robust preclinical models that faithfully capture the high inter- and intra-tumor heterogeneity of the human disease. Conventional cell lines lose the most common genetic abnormalities of glioblastoma (GBM), while primary cultured cells do not account for the influences of the microenvironment and the blood brain barrier on tumor biology and drug efficacy. These systems are also under strong selection pressure divergent from that *in vivo*, leading to reduced heterogeneity between cultured tumor cells and an overall shift away from *in vivo* characteristics. Here we describe a biobank of direct-from-patient derived orthotopic xenografts (GliomaPDOX) that preserve the diverse genetic and transcriptional landscapes found in human GBMs. A paired comparison between matched patients, GliomaPDOX, and short-term primary cell cultures revealed transcriptional changes associated with altered nutrient availability and non-tumor cell interactions, emphasizing the impact of the tumor microenvironment on *in vivo* gene expression. Further, GliomaPDOX models preserved signatures of differentiated brain cell types recapitulating the intratumor heterogeneity of non-stem and stem-like cells found in patient tumors. These results are in contrast to those found in gliomapshere culture systems, where signatures of differentiated brain cells are abolished. Collectively these data show that GliomaPDOX is a model system that preserves defining molecular and anatomical characteristics of GBM and is well-suited for translational research investigations.

TMOD-04. TARGETING A GLIOMA SPECIFIC lncRNA IN A HUMAN BRAIN ORGANOID TUMOR MODEL

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Long non-coding RNAs (lncRNAs) are transcripts >200 nucleotides long with essentially no protein coding potential. While certain lncRNAs play key roles in cancer and therefore represent an important class of therapeutic targets, very few lncRNAs have been studied in glioma. Here, we show that knockdown of a primate-specific lncRNA – referred to as *GTT1* – selectively inhibits the growth of both adult and pediatric glioma but does not adversely affect the viability of normal human glia and neurons. In a recent genome-scale CRISPR interference (CRISPRi)-based screen, we identified *GTT1* as one of 65 lncRNA genes that modify the growth of U87 glioblastoma cells. To prioritize lncRNAs for further study, we next performed a CRISPRi-based screen to identify lncRNA targets that also sensitize tumor cells to radiation. Both CRISPRi-mediated and antisense oligonucleotide (ASO)-mediated knockdown of *GTT1* inhibited the propagation of U87 cells in culture, and this effect synergized with radiation treatment. Furthermore, *GTT1* knockdown inhibited the growth of patient-derived glioma cells including adult glioblastoma and pediatric diffuse intrinsic pontine glioma (DIPG) in culture. Although *GTT1* is expressed in normal human brain, knockdown of this lncRNA in normal human astrocytes, cortical neurons and fetal forebrain tissues did not reduce cell viability. As *GTT1* is primate-specific, knockdown of this lncRNA in mouse xenograft models would not fully assess potential adverse effects. We therefore developed a human brain organoid model comprised of mature astrocytes and functional neurons. Patient-derived glioma cells engrafted into these organoids, and tumors grew in an infiltrative manner. *GTT1* knockdown in this brain organoid model strongly impaired tumor growth but did not reduce organoid viability. These studies identify *GTT1* as a glioma-specific therapeutic target and illustrate how this human brain organoid tumor model can be used to rapidly evaluate the tumor-specificity of novel therapeutics.

TMOD-05. GLIOMA-261 LUCIFERASE-EXPRESSING CELL LINE STIMULATES AN IMMUNOGENIC RESPONSE SIGNATURE IN AN IMMUNOCOMPETENT MURINE MODEL

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Immunocompetent murine models of glioma, such as GL261, are essential to assess immune therapy efficacy. The GL261 cell line expressing firefly luciferase (GL261RFluc), has been used to enable non-invasive tumor monitoring in preclinical studies. However, it is unclear if the GL261 and GL261RFluc cells are equivalent, particularly when applied to tumor immunology. C57BL/6 mice (n=20 per group, repeated once) underwent stereotaxic, intracranial implantation with GL261 or GL261RFLuc cells at 5x10^4cells/5uL and were assessed for survival. GL261 and GL261RFluc cell lines were assessed by CFSE proliferation assay. TGF-Beta2 ELISA and proteome profiler immunoassay were performed on equivalent cell culture lysates for cytokine analysis. Mice were implanted with GL261 or GL261RFluc cell lines, sacrificed at day 10, and brains were either disassociated, FACS stained, and sorted for immune cell surface antigens (n=4 each group) or formalin-fixed and paraffin embedded for immunohistochemistry (IHC, n=6 each group). Median survival for GL261 implanted mice was 20.9 ± 1.3 days with all animals progressing to a moribund state, while median survival was not reached for animals implanted with GL261RFluc cells (P=0.001). Quantitative IHC analyses of brains implanted with GL261RFluc cells showed significant increases in CD4 and CD8 cells but decrease in FoxP3 positive cells. Proliferation assays were equivalent. TGF-Beta2 ELISA showed significantly elevated levels in the GL261RFLuc cells. Proteomic profiler results demonstrated differential cytokine expression greater than 2-fold for over 25% of cytokines evaluated. The detected increases in chemoattractants CCL2 and CCL5 were particularly pronounced in GL261RFLuc cells. FACS analysis showed a trend of increased PD-L1 positive cells in brains implanted with GL261 cells, but this did not reach significance. GL261RFluc cells create an inflammatory immune microenvironment when implanted intracranially in C57BL/6 mice compared to GL261 cells. These findings suggest that investigators should avoid GL261RFluc cells when evaluating immune therapeutics in a C57BL/6 background.

TMOD-06. HIGH INCIDENCE OF TUMORS AFTER TREATMENT WITH A DNA-ALKYLATING AGENT IN MOUSE STRAINS COMMONLY USED IN PRE-CLINICAL STUDIES

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Glioblastoma (GBM) is commonly treated with the DNA-alkylating agent temozolomide (TMZ). Silencing O6-methylguanine-DNA methyltransferase (MGMT) by promoter hypermethylation predicts response to TMZ. Oral TMZ administration also affects tissues with low MGMT expression, such as bone marrow, resulting in high incidence of leukopenia and secondary leukemia in humans. Here we assessed the anti-tumor effect of TMZ in a panel of orthotopic GBM patient-derived xenografts (GBM-PDXs), and looked for evidence of secondary malignancy in the GBM-PDX lines with extended survival post-TMZ treatment, and also examined TMZ-treated naïve mice from two immunocompromised strains. First, a panel of 12 orthotopic GBM-PDX in athymic nude mice (NCRNU-F) were treated with 2-cycles of TMZ (40mg/kg/day for 5 days in a 21-day cycle) or with vehicle control gavage (n=10/treatment group). Treatment response was measured by log-rank test comparison of Kaplan-Meier survival curves. The untreated controls and TMZ-resistant PDXs succumbed to brain tumor burden immediately after treatment completion. TMZ-responsive GBM-PDX lines and GBM-naïve nude mice developed respiratory dysfunction 3–5 months after TMZ treatment. Histopathological analysis of lung tissue at autopsy revealed multiple foci of papillary adenocarcinoma (negative for human markers) in both lungs, at an incidence of 95% at 280 days in TMZ-treated nude mice, with no evidence of tumors in other organs. GBM-naïve untreated nude mice in the control group did not develop tumors after 1 year. TMZ treatment of severe combined immunodeficiency (SCID) mice resulted in 77% tumor incidence in the thymus and/or lungs in a GBM-PDX line, 3–4 months post-treatment, with a similar incidence for GBM-naïve treated mice, and no tumors observed in control mice after 9 months. These results present additional evidence of the tumorigenic potential of TMZ, particularly in the background of immunodeficiency. The development of lung/thymus tumors in TMZ-responsive GBM-PDX lines is an undesirable confounding factor not previously reported.

TMOD-07. LOCALIZATION OF ERLOTONIB RELATIVE TO MRI-BASED TUMOR EXTENT IN PDX GLIOBLASTOMA MODEL: TOWARDS A MATHEMATICAL MODEL FOR THE INTERFACE BETWEEN MRI AND DRUG DISTRIBUTION

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Clinical neuro-oncology relies on the hyperintensity of gadolinium (Gd) contrast agent on magnetic resonance imaging (MRI) in tumor regions to confirm that the blood-brain barrier (BBB) is locally compromised. While the extent of Gd hyperintensity may indicate that systemically administered drug is being distributed to the tumor regions, little is known about how a drug is distributed and how it may relate to the Gd hyperintensity. Add-

itionally, glioblastomas (GBMs) are diffusely invading neoplasms with a significant fraction of the overall tumor cells spread peripheral to the Gd abnormality, which raises uncertainty as to how or if the rest of the diffuse tumor is affected by drug. Given the gap in understanding drug delivery to the brain, we propose a quantitative approach to model drug delivery in GBM based on MRI and matrix-assisted laser desorption/ionization mass spectroscopy imaging (MALDI). T2-weighted (T2) and T1-weighted with Gd contrast (T1Gd) MRI images were acquired for an animal with the GBM12 orthotopic GBM patient derived xenograft (PDX) line dosed with 100mg/kg erlotinib. A T1Gd region of interest (ROI) captured the Gd-associated hyperintensity. MALDI was performed and aligned with MRI images. A Drug ROI was created to represent the increased intensity of erlotinib on MALDI images. Since the Drug ROI encompassed the T1Gd ROI, we subtracted the two ROIs to create a 'Drug No T1Gd' ROI, which represented the drug region beyond the edge of the T1Gd ROI. A 'Brain ROI' was created to represent the region of the brain outside of the drug's spread. The MALDI intensities for the three ROIs were all significantly different ($p<0.05$), with the T1Gd ROI having the highest mean, followed by the Drug No T1Gd and the Brain ROIs. By developing a quantitative understanding of drug distribution, we can make more robust predictions regarding treatment efficacy in the clinical setting.

TMOD-08. GROWTH IMPAIRMENT UNDER CONDITIONS FAVORING MITOCHONDRIAL OXIDATIVE METABOLISM IN A YEAST MODEL OF CANCER-ASSOCIATED ISOCITRATE DEHYDROGENASE MUTATION

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The use of the budding yeast *Saccharomyces cerevisiae* as a model system to study cancer allows for faster, more efficient elucidation of various molecular mechanisms, including mutation rate by fluctuation analysis, cell cycle analysis by flow cytometry, metabolism via growth rate analysis, and functional genomics via genomic array screening. The vast majority of low grade gliomas (LGGs) carry somatic mutations in isocitrate dehydrogenase 1 *(IDH1*) and 2 (*IDH2*) genes. *IDH1* and *IDH2* catalyze the oxidative decarboxylation of isocitrate to a-ketoglutarate (a-KG) in an $NADP^+$ dependent manner. A point mutation (R132H in IDH1 and R172H in IDH2) confers the neomorphic ability for the enzyme reduce a-KG to D-2-hydroxyglutarate (D2-HG). In *S. cerevisae*, the $NADP^+$ dependent isocitrate dehydrogenases are encoded by three different genes, *IDP1, IDP2* and *IDP3*. We have successfully generated a yeast model that carries the analogous mutation in the yeast IDP1 gene ($IDP1^{R148H}$). The allele was inserted at the HO locus, which does not alter the endogenous *IDP1* gene. In this way, the resulting strain carries both a wild-type and mutant allele of *IDP*, more closely mimicking the metabolic state of glioma cells. We have validated this insertion by PCR, sequencing, and tetrad analysis. The production of the mutant $IDP1^{R148H}$ protein was detected by Western blot. The $IDP1^{R148H}$ strain shows normal growth on glucose and galactose-containing solid media, but reduced growth on glycerol-containing solid media compared to parental or $IDP1^{WT}$ strains. Impaired growth of yeast when glycerol is the sole carbon source suggests a defect in mitochondrial oxidative metabolism. This observation is consistent with a previous yeast $IDP1^{R148H}$ model which showed extensive mitochondrial DNA loss and respiration defects. Taken together, we have developed a model of IDH-mutant LGGs in *S. cerevisiae* that can be further utilized to study molecular mechanisms underlying tumorigenesis of LGGs.

TMOD-09. TARGETING THE PI3K-mTOR PATHWAY AND ELUCIDATING MECHANISMS OF RESISTANCE IN A NOVEL AND RELEVANT ANIMAL MODEL OF GLIOBLASTOMA

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Pediatric glioblastoma (pGBM) and adult glioblastoma (aGBM), henceforth collectively referenced as GBM are incurable brain tumors with variable prognosis and response to treatments due to the intermolecular heterogeneity. In particular, the GBM MYCN subtype is a highly aggressive genetic group in both pediatrics and adults where patients have a dismal median survival of only 14 months. Furthermore, this subtype is enriched with loss of the tumor suppressor genes TP53 and PTEN, leading to PI3K-AKT pathway activation and DNA-checkpoint abnormalities. Here, we report the generation of a novel syngeneic GBM mouse model of the MYCN subtype. We isolated and transduced C57BL/6 Sox2-CRE neural stem cells (NSCs) with inverted retroviral-cassettes of the murine Mycn oncogene and shRNA targeting tumor suppressor genes p53 and Pten. The retroviral-cassettes are flanked by tandem LoxP sites arranged so that CRE recombinase expression inverts the cassettes in frame allowing for MYCN protein expression and loss of the P53 and PTEN proteins. Transgene activation is accompanied with selectable cell surface markers and fluorescent tags enabling for fluorescent activated cell sorting (FACS) of desired cell populations. MYCN protein expression with concomitant silencing of P53 and PTEN protein leads to increased proliferation and formation of invasive high-grade gliomas when implanted into the frontal cortex of immune competent C57BL/6 mice and NOD-SCID mice. Using several next generation brain penetrant small molecule inhibitors of the PI3K-AKT pathway, we show tumor regression *in vivo*. Moreover, we have identified several novel mechanisms of PI3K-AKT treatment resistance and are currently identifying therapies that may overcome this resistance. In summary, well defined genetic drivers of GBM can lead to informed mouse model generation to test promising therapies.

TMOD-10. METABOLIC AND BLOOD-BRAIN BARRIER MARKERS FOR FLUORESCENCE-GUIDED SURGERY: SYSTEMATIC HIGH-RESOLUTION MICROSCOPY INVESTIGATION IN HUMAN RELEVANT EXPERIMENTAL GLIOMAS

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BACKGROUND. Fluorescence guidance with 5-aminolevulinic acid(5-ALA) increases resection extent and may benefit surgical outcomes in high-grade gliomas. We investigated if a combination of various fluorescence guidance techniques can provide further advantage. We systematically assessed and compared the fluorescent patterns of 5-ALA-induced protoporphyrin IX (PpIX), fluorescein sodium(FNa) and indocyanine green(ICG) to identify GL261 gliomas in mice and RFP-U251 gliomas in rats. METHODS. 5-ALA(5mg), FNa(5 mg/kg) and ICG(20mg/kg) were administered perioperatively. Fluorescence patterns were recorded with operative microscope, laser scanning confocal microscope, and confocal laser endomicroscope. Fluorescence was assessed quantitatively as a surface area of fluorescent positive tissue and qualitatively(false/true, positive/negative) as compared to HE-stained histology. RESULTS. FNa highlighted a larger surface area($18.0mm^2$) than white light($16.9mm^2$,$p=0.016$) or PpIX ($16.0mm^2$,$p=0.035$). Both 5-ALA and FNa showed inhomogeneous staining patterns: multiple areas of equal staining, when PpIX was present and FNa was not, and vice versa. ICG was visible in 8/31 tumor samples, all immediately after injection. ICG did not reveal clear tumor margins, but stained non-tumor tissue and nearby vasculature. FNa signal was stronger (tumor to background ratio (TBR)1.93 ± 0.56) compared to 5-ALA(1.52 ± 0.31;p CONCLUSION. ICG highlighted tumor only within the first few minutes and stained mostly hypervascularized areas. Confocal and surgical imaging revealed inhomogeneous tumor border staining with PpIX/FNa. Simultaneous administration of 5-ALA and FNA may provide additional benefit. Neither ICG, 5-ALA or FNa worked perfectly, emphasizing the need for more specific markers for fluorescence-guided brain tumor resection.

TMOD-11. IMAGING BASED INVASION METRIC PREDICTIVE OF RESPONSE TO ABT414 IN ORTHOTOPIC EGFRviii AMPLIFIED PATIENT DERIVED XENOGRAFTS

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BACKGROUND: Failed trials involving targeted therapies face a daunting task of understanding whether the root cause was inadequate targeting, resistance, or insufficient delivery to the tumor. Previous work with a biomathematical model has shown the prognostic value of an imaging based invasion metric, D/ρ (mm^2/yr), which is linearly correlated with the extent of tumor burden beyond the imaging abnormality. As this extent likely impacts the definition of sufficient drug delivery, we investigated whether this metric is able to predict response to ABT-414, an antibody drug conjugate targeting EGFR. METHODS: *Preclinical experiments:* After initial screening *in vitro* and in flank, the efficacy of ABT-414 was evaluated for three patient derived cell lines (PDXs), GBM6, GBM12, and GBM39, implanted orthotopically. All three showed strong response to ABT-414 in the previous experiments. In the orthotopic setting, GBM39 was very sensitive to therapy (> 155 days benefit), GBM12 was moderately sensitive (15–30 days benefit), and GBM6

was resistant to therapy (no benefit). *Invasion Index:* D/ρ was calculated for each cell line based on the original patients' pre-treatment T1-weighted with Gd contrast and FLAIR MRIs. We then analyzed whether D/ρ was correlated with the observed response to ABT-414. RESULTS: GBM6 had the highest D/ρ = 4.7 (greatest proportion of tumor cells invaded beyond the imaging abnormality), GBM12 was in the middle with D/ρ = 1.7, and GBM39 had the lowest D/ρ = 0.9. This perfectly (inversely) correlates with the response patterns. GBM6 showed no response, GBM12 had a moderate response, and GBM39 showed the greatest response to ABT-414. CONCLUSION: Our results suggest that the imaging based tumor invasion metric, D/ρ, is inversely correlated with tumor response to ABT-414. This supports our hypothesis that drugs with low BBB permeability will be more beneficial for tumors with little tumor burden beyond the imaging abnormality.

TMOD-13. A NOVEL 3D HIGH-RESOLUTION HISTOPATHOLOGICAL IMAGE RECONSTRUCTION METHOD VERSUS COMMON 2D AND 3D IMAGING METHODOLOGIES FOR APPLICATION IN CANCER SPHEROID RESEARCH: WHICH IS BETTER?

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BACKGROUND: Three-dimensional tumour spheroid models are increasingly used in cancer research. However, imaging for quantification analysis of spheroids in drug testing has remained primarily based on 2D methodologies. Imaging 3D structures in 2D can cause discrepancies and introduce interpretive bias. We previously described a novel method to create high-resolution 3D spheroid images using histopathological sectioning. We compare for the first time 3D histopathological images and data analysis of glioma spheroid models in drug studies to commonly used imaging techniques, highlighting the advantages of 3D imaging over 2D methodologies. METHODS: U251 glioma spheroids embedded in collagen were untreated or treated with inhibitors 6-Bromoindirubin-3'-oxime (BIO) or MI-192. Spheroids were imaged at 24-hour time points over 72 hours using light-microscopy and confocal laser-microscopy and then subsequently embedded in paraffin and sectioned at 5µm. Slides were H&E stained and serially scanned at 20x magnification. Custom software was used to digitally reconstruct the spheroid in 3D. For details at the single cellular level, 70µm spheroids were also imaged at 72 hours at high-magnification using an Instant Structured illumination microscope (iSIM). RESULTS: Superior detail of core cellular components and migratory single cells was achieved by 3D histopathological reconstruction compared to all other methods. Migration indices were similar to 3D confocal-generated reconstructions demonstrating promoted migration with MI-192 and reduced migration with BIO. Detailed morphological analysis of migratory cells was achieved at 40x optical magnification using our 3D histology method. However, greater morphological detail was obtained using high-magnification iSIM. Failure to capture migratory cells in the z-plane using 2D techniques lead to considerable differences between 3D and 2D imaging data. CONCLUSION: We have shown that 3D histopathological image reconstruction is a preferable method for cell migration and morphological analyses of 3D cancer spheroid models. This method can be used for high-resolution analysis of both core and migratory cells.

TMOD-14. A PATIENT-DERIVED CANCER CELL LINE ATLAS OF PRIMARY AND METASTATIC CENTRAL NERVOUS SYSTEM TUMORS

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BACKGROUND: Tumors involving the central nervous system (CNS) include over 200 primary and metastatic subtypes with major clinical impact. Research on CNS neoplasms has been hampered by the lack of appropriate models for many subtypes. We established a robust workflow and systematic culturing approach to create cancer cell line models from all adult and pediatric CNS cancer patients and here report the results of these ongoing efforts. METHODS: Tumor samples from consented patients with CNS cancers were systematically collected from 2008–18. Tumors were grown in different media and substrates and growth verified by >5 passages. Genomic verification was performed using NGS analysis (focused SNV and CNA) and expression profiling assays. RESULTS: We attempted to generate cell line models from >1500 consented brain tumor patients at the Brigham and Women's and Boston Children's Hospital (IRB-10–417) under the DFCI Living Tissue Bank Program as well as within the Broad Institute Cancer Cell Line Factory (CCLF) Project and the Leuven Living Tissue Bank (IRB-S59804, Belgium). 120 different tumor types, including high and low-grade brain tumors of both adult and pediatric origin, were evaluated for *in vitro* growth in >2000 culturing attempts. The success rate of growing high-grade tumors (i.e. glioma and of other origin) was robustly high (~50%), while the growth verification rate for low-grade tumors remained low (<1%). Overall, growth beyond passage 5 was achieved in ~30% of cases, and of the growth verified models, also ~30% were genomically verified to represent cancer, with the majority maintaining genomic and/or transcriptional features. In addition to primary CNS tumors, we were now also able to grow metastatic cultures, resulting in >150 novel model systems covering >20 primary and metastatic diagnoses. CONCLUSION: Patient-derived cell lines may be created at scale from primary and metastatic CNS tumors to support preclinical cancer research but technological improvements will be required to culture even more tumor types.

TMOD-15. RELIABILITY OF IMAGING-BASED MEASURES OF TUMOR 'MASS-EFFECT'– EVIDENCE FROM A COMPUTATIONAL STUDY

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Elevated tumor mass-effect is associated to poor prognosis in GBM [1,2]. However, tumor mass-effect is poorly quantified in clinical practice. Recently, Steed et al. [2] proposed 'Lateral ventricle displacement' (LVd), defined as the change in center-of-mass position of the lateral ventricles between an undeformed reference and the tumor-bearing anatomy, as quantitative imaging measure of mass-effect. They found that the magnitude of LVd in GBM patients can be associated with overall survival. These results show the clinical importance of tumor mass-effect in GBM, warranting robust clinical measures. This study characterizes image-derived estimates of tumor mass-effect by their ability to measure mass-effect accurately and reliably. We use a mathematical model to simulate tumor growth, which allows us to control and objectively quantify 'mass-effect' [3]. For given simulation parameters and growth location, we compute estimates of mass-effect from anatomical deformation during the growth process. We use multiple regression analysis to evaluate the ability of different estimates to explain the tumor's objective mass-effect, measured by the tumor-induced pressure on the skull. Preliminary results from tumor growth simulations at 15 locations across a normal brain atlas confirm the potential of LVd as predictor of tumor mass-effect (R^2=0.98). The maximum left-right displacement along the midline in a selected plane, a measure for 'midline shift', only explains a smaller percentage of mass-effect variability in the simulated population and strongly depends on the measurement plane (R^2=0.20–0.85). We will report results of an extended study that applies our analysis approach to a large and diverse population of approximately 100 simulated tumors, by varying growth location and parameters related to the tumor's proliferative and invasive potential. References: [1] Gamburg et al. IJROBP, 2000, 48, 5: 1359–62, [2] Steed et al. Scientific Reports, 2018, 8: 2827, [3] Abler et al. Neuro-Oncology, 2017, 19, suppl 6: vi245.

TMOD-16. COMPARING THE EFFECTS OF CIRCUMSCRIBED VERSUS INFILTRATIVE TUMOR GROWTH PATTERNS ON FUNCTIONAL CONNECTIVITY, HEMODYNAMIC PARAMETERS, AND BEHAVIOR IN A MOUSE GLIOMA MODEL

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BACKGROUND: Gliomas can cause significant changes in normal brain function leading to behavior deficits. Functional connectivity (fc) disruptions may contribute to these deficits. However, investigating the effects of glioma growth on fc in humans is complicated by heterogeneity in lesion size, type, and location across subjects. In this study, we evaluated the effects of different patterns of growth on fc, hemodynamic parameters, and behavior in a controlled mouse glioma model. METHODS: $5x10^4$ glioma cells growing in either a circumscribed (U87 PDE7B H217Q, n=13) or infiltrative (U87 PDE7B WT, n=14) pattern were stereotactically injected into the forepaw somatosensory cortex of adult nude mice. We monitored tumor burden with weekly bioluminescence imaging (BLI). Additionally, we evalu-

ated fc with weekly functional connectivity optical intrinsic signal imaging (fcOIS). Fc was calculated between homotopic brain regions. Furthermore, cerebral blood flow (CBF) and hemodynamic lags were assessed. Lastly, we assessed performance on a somatomotor behavior battery during the 7th week post-injection. RESULTS: Mice with infiltrative tumors had more tumor burden then mice with circumscribed tumors. Furthermore, infiltrative tumors disrupted fc and produced hemodynamic lags earlier than circumscribed tumors. However, infiltrative tumors didn't change CBF, while circumscribed tumors increased CBF near the injection site. Lastly, mice with infiltrative tumors exhibited somatomotor deficits, while mice with circumscribed tumors performed similar to sham mice (n=20). CONCLUSIONS: We were able to detect differences in fc disruptions in mice with gliomas growing in different patterns with fcOIS. Infiltrative tumors produced greater tumor burden and fc disruptions than circumscribed tumors. Additionally, hemodynamic lag was a better indicator of tumor burden than CBF. Lastly, infiltrative tumors induced behavior deficits during this study, while circumscribed tumors did not. A better understanding of how fc disruptions contribute to behavior deficits in glioma patients may lead to better patient risk stratification and improved behavior outcomes.

TMOD-17. A NOVEL ADENOVIRAL-PERMISSIVE, IMMUNOCOMPETENT HAMSTER MODEL TO EVALUATE ONCOLYTIC ADENOVIRAL THERAPY FOR GLIOBLASTOMA

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Treatment with the oncolytic adenovirus, Delta-24-RGD, resulted in dramatic tumor response in 20% of recurrent malignant glioma patients in a recent Phase I clinical trial. Studies in immunocompetent mice corroborate the patient data indicating that Delta-24-RGD effects are due to both direct tumor cell lysis and viral-mediated anti-tumor immune response. However, it is unclear why only a fraction of patients responds in this manner. Due to poor adenoviral replication in immunocompetent mouse models, the mechanisms by which Delta-24-RGD elicits an effective anti-tumor immune response remain poorly understood. Therefore, we sought to develop a syngeneic Syrian hamster glioma model that is both adenovirus replication-permissive and immunocompetent. We transformed hamster neural stem cells with hTERT, simian virus 40 large T antigen, and h-RasV12 and re-implanted the transformed cells into hamster brains where they developed into tumors. Hamster glioma stem cells (GSCs) were isolated from the resulting tumors and were re-implanted into naive hamster brains using a guide-screw system. *In vitro*, hamster GSCs supported viral replication and were susceptible to Delta-24-RGD mediated cell death. *In vivo*, hamster GSCs consistently developed into highly proliferative tumors resembling high-grade glioma. Following delivery of Delta-24-RGD by intratumoral injection, immunohistochemistry for viral proteins demonstrated viral infectivity and replication in hamster gliomas. Flow cytometric analysis of hamster gliomas revealed increased T-cell infiltration in Delta-24-RGD infected tumors. Delta-24-RGD treatment of tumor-bearing hamsters led to significantly increased survival compared to hamsters treated with PBS. In summary, we have developed an adenovirus-permissive, immunocompetent hamster glioma model that provides a novel platform in which to study the interactions between tumor cells, the host immune system, and oncolytic adenoviral therapy.

TMOD-18. THE PATIENT DERIVED XENOGRAFT NATIONAL RESOURCE: A COMPREHENSIVE COLLECTION OF HIGH-GRADE GLIOMA MODELS FOR PRE-CLINICAL AND TRANSLATIONAL STUDIES

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Patient derived xenograft (PDX) models have shown great utility for pre-clinical and translational studies for a range of malignancies. We have established a comprehensive, publically available collection of 95 high-grade glioma flank PDX models. Viable PDX were derived from Glioblastoma, IDH-wildtype (n=91), Glioblastoma, IDH-mutant (n=2), Diffuse Midline Glioma, H3 K27M-mutant (n=1), and Anaplastic Oligodendroglioma (n=1) and include both primary (n=60) and recurrent (n=35) tumors. Comprehensive molecular characterization of PDX is ongoing and, to date, has included whole exome sequencing (WES, n=82), RNA-sequencing (n=40), and genome-wide methylation profiling (n=78) that included MGMT promoter, with data available in cBioPortal. PDX reflected the genetic characteristics of glioblastoma, with the majority harboring *TERT* promoter mutations, chromosomal gain +7, loss -10 and homozygous deletion of *CKDN2A/B*. *EGFR* alterations were frequent (~40%) and included amplification, point mutation, EGFRvIII, and other splice variants. Other common alterations, including amplifications of *MET*, *CDK4*, *CDK6*, *MDM2*, *MDM4* and mutation of *TP53*, *PTEN*, *NF1*, *RB1*, *PIK3CA*, *PIK3R1* were present at similar frequencies reported by TCGA, with the exception of *PDGFRA* alterations that were underrepresented in PDX. RNA-sequencing showed representation of each of the glioblastoma gene expression subtypes. To assess preservation of genetic features during xeongrafting, we performed WES on 20 matched patient tumors. The vast majority of genetic driver alterations were shared between patient and PDX, including *EGFR*, EGFRvIII, *CDK4*, *MDM2*, and *MET* amplifications. In 3 PDX, subclonal selection events were observed, including amplifications of *MYCN*, *CDK6* and an *EGFR* splice variant, as well as selection against *PDGFRA* amplification. Overall, our large panel of PDX models reflects the genetic heterogeneity of glioblastoma and largely preserves the genetic features of the primary patient tumors. The PDX National Resource is a powerful tool for neuro-oncology research, with all PDX models and genomic data openly available (http://www.mayo.edu/research/labs/translational-neuro-oncology/mayo-clinic-brain-tumor-patient-derived-xenograft-national-resource).

TMOD-19. MUTANT IDH1 PROMOTES GLIOMA FORMATION IN VIVO

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Gliomas are the most common primary central nervous system tumor but the molecular mechanisms responsible for their development and progression are not completely understood. *Isocitrate dehydrogenase 1 (IDH1)* is the most commonly mutated gene in grade II-III glioma and secondary glioblastoma (GBM) but functional validation of this alteration has been hampered by difficulties generating autochthonous mouse models harboring mutant *IDH*. In this study, we used the RCAS/TVA glioma mouse model to assess the role of $IDH1^{R132H}$ in glioma development in the context of clinically-relevant cooperating genetic alterations *in vitro* and *in vivo*. Immortal *Cdkn2a*, *Pten*, and *Atrx*-deficient astrocytes expressing $IDH1^{R132H}$ exhibited elevated (R)-2-hydroxyglutarate (2-HG) levels, reduced NADPH, increased proliferation, and anchorage-independent growth. Cell proliferation and soft agar growth was significantly enhanced by co-expression of PDGFA. Substitution of $IDH1^{R132H}$ with a cell membrane-permeable [tri-fluoromethyl benzyl (TFMB)-esterified] version of (*R*)-2HG mimicked the phenotype observed in cells expressing $IDH1^{R132H}$. Interestingly, addition of TFMB-(*R*)-2HG further enhanced colony formation in cells expressing $IDH1^{R132H}$ while treatment with the mutant IDH1 inhibitor AG-120 reduced anchorage independent growth. These data suggest that the effects of $IDH1^{R132H}$ are mediated by 2-HG and not loss of native IDH1 activity. Although not sufficient on its own, $IDH1^{R132H}$ cooperated with PDGFA and loss of *Cdkn2a, Atrx,* and *Pten* to promote glioma development *in vivo*. These tumors resembled proneural human mutant *IDH1* GBM genetically, histologically, and functionally. Our findings support the hypothesis that $IDH1^{R132H}$ promotes glioma development through production of 2-HG. This model validates a role for $IDH1^{R132H}$ in glioma development, permits mechanistic studies that further our understanding of oncogenesis in this context, and enables rapid testing of rationale therapeutic strategies designed to combat this deadly disease.

TMOD-20. TRKing DOWN NOVEL THERAPEUTIC TARGETS IN GLIOMAS

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The occurrence and importance of gene fusions in glioma has been appreciated only recently, largely due to high-throughput technologies, and gene fusions have been indicated as one of the major genomic abnormalities in glioblastoma (GBM), the most frequent and aggressive glioma subtype. The functional role of the majority of these alterations is completely unexplored. Recurrent gene fusions involving the Tropomyosin Receptor

tyrosine Kinases (TRK) receptor family have been recently described in a variety of tumors, including both pediatric and adult low-grade (LGG) and high-grade gliomas (HGG). Strikingly, 40% of non-brain stem pediatric HGGs in infants have been shown to carry TRK gene fusions, making Trk inhibition an important potential therapeutic intervention in patients in which the current treatment modalities have devastating side effects. For the *in vivo* study of genomic rearrangements, we have recently generated an innovative mouse model that combines the genome editing capability of the CRISPR/Cas9 system with the somatic gene delivery of the RCAS/tv-a system. By searching the scientific literature we have identified approximately 30 different fusion involving NTRK1, NTRK2 and NTRK3 genes. To discriminate those fusions with tumorigenic potential in gliomas we have performed an *in vivo* gRNA pair screening. Four different RCAS-gRNA library pools have been transduced into *p53-null* TVA-Cas9 neural stem cells (NSCs) and subsequently transplanted into *NOD/SCID* mice. So far, half of the mice rapidly developed (1–2 months) quite aggressive tumors. We are currently characterizing those tumors to identify the most potent oncogenic NTRK fusions. NTRK gene rearrangements are emerging as novel targets across multiple tumor types, because of the increasing availability of new drugs with anti-Trk activity. As part of our studies, we are investigating the response and the resistance mechanism to Trk inhibitors that are currently used in different clinical trials.

TMOD-21. NEURO-ONCOLOGICAL ANIMAL MODELS AND PERITUMOURAL OEDEMA - IN THE BLIND SPOT?

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Brain tumour associated oedema is a significant contributor to patient morbidity and mortality whilst striving for disease control. Indeed initially, cerebral oedema may result in the signs and symptoms leading to patient presentation rather than the tumour itself. For several decades, Dexamethasone has remained the mainstay in treatment notwithstanding its host of side effects. As such there is growing interest for alternative agents. A prerequisite for the development of novel therapeutic agents would be an animal model with a high degree of validity. Great progress has been made with animal models of brain tumours but a paucity of focus on peritumoural oedema. We sought to investigate animal models of brain tumour that exhibit oedema which can be confirmed on imaging and that manifest clinically. A systematic scoping review of EMBASE, CINAHL, Medline and Pubmed identified 603 reports that matched initial broad inclusion criteria, however as yet, most models did not fully satisfy our search for the ideal model. Further work is required to define an animal model that consistently demonstrates peritumoural oedema that is radiologically quantifiable and clinically manifest to allow effective research into alternative oedema-supressing agents.

TMOD-22. MODELING SEX DIFFERENCES IN p53 GAIN-OF-FUNCTION MUTATIONS IN GLIOBLASTOMA

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The tumor suppressor *TP53* (p53) is the most commonly mutated gene in cancer and is one of the most frequently mutated genes in glioblastoma (GBM). The most common p53 mutations are missense mutations in the DNA binding domain that lead to the expression of full length mutant p53 protein. In addition to the loss of tumor suppressor function, these mutations can endow oncogenic gain-of-function abilities that allow mutant p53 to bind and regulate the promoters of aberrant target genes, driving tumorigenesis. However, the mechanisms that control mutant p53 target gene specificity and the subsequent malignant phenotypes are poorly understood. We combined and analyzed patient mutation data from the COSMIC, TCGA and IARC databases to determine the prevalence of individual p53 mutations in CNS tumors. This revealed a subset of six missense mutations that exhibit significant sex differences in their frequency, suggesting that these mutations may have a sex specific effect on cancer cell fitness. Four codons were mutated more frequently in females: Y205 (*p=0.004*), D184 (*p=0.0172*), V216 (*p=0.0253*), and V272 (*p=0.0336*), and two codons were mutated more frequently in males: Y220 (*p=0.0104*) and R282 (*p=0.0496*). *We* developed a murine astrocyte model that will allow us to investigate the sex specific effect of each gain-of-function mutation on transcription and tumorigenesis. Using CRISPR/Cas9, relevant point mutations were inserted into the p53 DNA-binding-domain of male and female p53 heterozygous primary mouse astrocytes. These astrocytes express a single mutant p53 allele, and reflect the silencing of the WT p53 allele common in GBM. Using this model, we can directly compare the transcriptional activity of each gain-of-function mutation using ChIP-sequencing and RNA-sequencing. These cells also provide a unique model for mechanistic studies to determine the tumorigenic effects of each gain-of-function mutation, including proliferation, invasion, clonogenicity and *in vivo* tumorigenesis.

TMOD-23. DYNAMIC PATTERNS OF GLIOBLASTOMA CLONAL EVOLUTION IN RESPONSE TO CHEMORADIOTHERAPY

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Despite aggressive multimodal therapy, glioblastoma (GBM) remains incurable and inevitably relapses. Recent data have implicated intratumoral heterogeneity as the driver of therapy resistance and tumour relapse in GBM. Models that capture the evolution of GBM biology in response to standard-of-care (SoC) chemoradiotherapy will allow for the identification of cellular mechanisms that govern GBM therapy failure. In this study, we coupled cellular DNA barcoding technology with our novel patient-derived xenograft SoC model (combined temozolomide and radiation treatment) to profile the clonal evolution of GBM stem cells (GSCs) through therapy. We report the successful barcoding of patient-derived primary, treatment-naive GSCs at a single cell resolution that were expanded into clonal populations, intracranially engrafted in immune-deficient mice, and treated with SoC therapy. We performed MRI imaging to identify spatial recurrence patterns of GSCs through the *in vivo* chemoradiotherapy model. We then interrogated the temporal fate of clonal barcoded GSC populations through SoC therapy model to identify differential barcode selection in response to treatment. Through this, we determined dynamics patterns of a pre-existing or a therapy-driven GSC subpopulation(s) seeding GBM tumour relapse. Profiling the dynamic nature of heterogeneous GBM subpopulations through disease progression and SoC treatment may lead to the identification of the modes of therapy resistance utilized by GBM to drive disease relapse.

TMOD-24. PATIENT-DERIVED BRAIN TUMOUR IPSCS: MODELS FOR INVESTIGATING GLIOMA STEMNESS AND DRUG DISCOVERY

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BACKGROUND: Dysregulated, stem cell-like self-renewal has been implicated in glioma treatment resistance and tumour recurrence. Drugs that eliminate tumour cells possessing this malignant characteristic are urgently needed. It remains, however, an experimental challenge to link heterogeneous glioma genotypes to drug response at scale. To this end, we successfully derived patient-specific induced pluripotent stem cell (iPSC) models from both low- (LGG) and high-grade gliomas (HGG) and developed an initial drug discovery application. METHODS: Brain tumour tissue, acquired at surgery, was reprogrammed. Derived iPSC models were characterised using pluripotency markers, tri-germinal layer differentiation, gene expression, karyology and deep whole genome sequencing (WGS, iPSC versus parental tumour). Glioma iPSC differentiation in 2-dimensional (adherent, optically clear 96-well imaging plates) and 3-dimensional (organoid) culture was carried out. Gene expression of neural induction and neuronal differentiation was analysed using mRNA-seq. Neural cancer stem cells from each glioma iPSC line were orthotopically implanted in vivo. RESULTS: Reprogrammed cells were confirmed as fully-reprogramed/stable iPSCs, with mutational variants (SNPs, CNVs) preserved as compared to the parental tumours. Glioma iPSC maturation and quantification of TUJ-1 expression indicated a 'differentiation block' in the HGG iPSC models. This phenotype was concordant in HGG iPSC-derived tumour organoids which displayed SOX2/MKI67-positive neural rosettes. Consistently, mice developed xenograft tumours with GBM histopathological characteristics. Expression profiling during neuronal differentiation (from iPSC to neural stem cells to neurons) has revealed candidate genes that may be responsible for the phenotypic differences between HGG and control/LGG iPSC models. CONCLUSIONS: Our adherent, organoid and in vivo iPSC models may uncover genetic mutations and regulatory networks underlying glioma stem cell self-renewal capability, and provide a basis for industrial-scale drug discovery. Here we have successfully implemented the first stages towards this development (in a 96-well assay format).

Ultimately, our patient-derived iPSC-based approach may enable personalised precision medicine strategies against glioma.

TMOD-25. MODELING IDH1-MUTATED GLIOMAS: GENERATION, CHARACTERIZATION AND THERAPEUTIC SENSITIVITIES OF SEVEN PATIENT-DERIVED IDH1-MUTANT GLIOMA CELL LINES

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INTRODUCTION: In spite of significant attention IDH mutations have attracted in glioma research, *in vitro* model systems with endogenous IDH mutations remain scarce. Development of these models is crucial not only for unraveling the molecular mechanisms that contribute to this disease entity, but especially for the development of new therapeutic interventions. METHODS: Fresh glioma tissue was obtained directly from the operating theater, dissociated and cultured under serum-free conditions. The presence of IDH mutations was verified at multiple passages using Sanger sequencing. D2-HG levels were measured through mass spectrometry. Cell proliferation was assessed through cell counting, cell viability was measured with an ATP-based viability assay. Drug screens were carried out with an FDA-approved Oncology Drugs Set from the NIH and multiple IDH mutant-specific inhibitors. RESULTS: Over 800 consecutive low grade and high grade glioma samples were processed for cell culture. From seven tumors one or more successful IDH mutant cell cultures were established that maintain the mutation in culture for at least five passages. All cultures were derived from astrocytic tumors. From one patient both the primary grade II astrocytoma and recurrent grade IV glioblastoma formed successful IDH-mutant cell lines. IDH-mutant specific inhibitors showed modest effects on cell viability, indicating limited dependence on D2-HG for growth. Drug screening revealed a subset of compounds that decrease viability at clinically-feasible drug concentrations which warrant further investigation. Whole exome sequencing in parallel with matched fresh-frozen tumor tissue, as well as RNA sequencing analyses are underway. CONCLUSION: We established a set of cell cultures derived from seven IDH mutant gliomas and characterized these on both genetic and transcriptional levels, and investigated drug sensitivity and D2-HG dependence. This unique set can be utilized to investigate novel therapeutic strategies.

TMOD-26. CYTOMEGALOVIRUS PROMOTES GLIOBLASTOMA GROWTH VIA PDGF-D DRIVEN PERICYTE RECRUITMENT AND ANGIOGENESIS

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Human cytomegalovirus (HCMV) is highly prevalent, and like other herpes viruses, can persist for life in its host in a latent state. However, HCMV can be severely pathogenic in immunocompromised individuals. Interestingly, HCMV proteins and nucleic acids have been identified in up to 90% of patients with the incurable brain tumor glioblastoma (GBM) as well as some other cancers. Accumulating data supports the clinical relevance of HCMV in GBM, with some encouraging responses reported with HCMV-targeted immunotherapies. Although various HCMV proteins increase cell proliferation and invasion, a mechanistic link between HCMV and cancer *in vivo* has not been established, and the role of HCMV in GBM remains a subject of debate. In the current report we show that perinatal murine CMV (MCMV) infection induces a pro-angiogenic secretome, increasing tumor growth, pericyte accumulation, angiogenesis and tumor blood flow in a murine GBM model. Specifically, we identify platelet-derived growth factor-d (PDGF-D) as a CMV-induced factor essential for tumor growth. In our model, MCMV can be seen in tumor cells and vascular pericytes, a finding that we confirm in human GBM specimens. The anti-viral drug cidofovir improves survival in MCMV-infected mice, inhibiting MCMV activation, PDGF-D expression, pericyte recruitment and tumor angiogenesis. Together these data provide the first mechanistic explanation of how CMV potentiates GBM growth *in vivo*, identify PDGF-D as a potential therapeutic target, and support the application of anti-viral approaches for GBM therapy.

TMOD-27. HUMANIZED MICROBIOME MOUSE MODELS TO ENHANCE IMMUNOTHERAPY IN GLIOBLASTOMA

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Cancer immunotherapies, including the checkpoint inhibitors, demonstrate remarkable success in patients with melanoma, but have not shown a similar efficacy in patients with glioblastoma (GBM). Recently, the composition of the gut microbiome has been shown to promote resistance to immune checkpoint inhibitors in melanoma and other cancers suggesting a favorable composition of the gut microbiome is needed to produce an optimal response to checkpoint inhibitors and subsequent anti-tumor immune responses. We propose that the gut microbiome of GBM patients promotes resistance to immunotherapies. To investigate this, we have collected and analyzed the gut microbiome from GBM patients (short-term and long-term survivors) and healthy controls by microbial 16S and metagenomic sequencing. These results will determine microbiome differences between GBM patients and controls, as well as differences within each patient as the disease progressed. Furthermore, all GBM pre-clinical studies to date have been performed in mouse models using *mouse* gut microbiota. We have previously found that human fecal samples can be transplanted into gnotobiotic (germ-free) mice to successfully colonize the mouse GI tract with human microbes. Herein, we have established humanized microbiome mice utilizing human GBM donor fecal samples previously obtained and analyzed. This model is critical because it allows us to study the relationship between the human microbiome among different GBM patients, and the responsiveness to therapies using the syngeneic GL261 intracranial model. We are currently using these humanized microbiome mouse models to analyze pre-clinical testing of anti-PD-1 in GBM, and endpoints to assess efficacy will include survival times, tumor growth, and examining the periphery and tumor environment for phenotype(s) of infiltrating immune cells. These studies will enhance our understanding of the mechanism of GBM patient's microbiome in the resistance to immunotherapies and lead to new therapeutic strategies to alter the microbiome composition to enhance immunotherapy for GBM.

TMOD-28. MYC OVEREXPRESSION DRIVES MEDULLOBLASTOMA FROM HUMAN NEUROEPITHELIAL STEM CELLS

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Medulloblastoma (MB) is the most prevalent malignant brain tumor in children. Based on molecular genetic profiling, this disease can be classified into four major subgroups which display distinct clinical features. Group 3 MBs are associated with overexpression or amplification of the *MYC* oncogene and rarely show any mutations in the tumor suppressor protein p53. Patients with MYC-driven MB have a particularly high risk of recurrence and are associated with extremely poor prognosis. Thus, modeling MYC-driven MB is critical for the development and testing of potential new treatment approaches for these high-risk MBs. Here we show the first human MB model developed from human hindbrain neuroepithelial stem (NES) cells and induced pluripotent stem cell-derived NES (iPS-NES) by lentiviral overexpression of wild-type MYC. Following orthotopic transplantation into immunodeficient mice these embryonic cells generate aggressive brain tumors with high penetrance in the absence of p53 mutations. The MYC-driven tumors are comprised of poorly differentiated cells with high expression of the proliferation marker Ki-67. Tumors also express early neuronal lineage marker Tuj-1 (neuron-specific class 3 beta tubulin) and the transcription factor OTX2. All these features closely mimic those of human Group 3 MB. The establishment of these human MYC-driven MB animal models will facilitate the functional study of MB biology and testing of potential therapies.

TMOD-29. MOLECULAR CHARACTERIZATION OF GLIOMA PATIENT-DERIVED ORTHOTOPIC XENOGRAFTS: FROM BASIC RESEARCH TO PRECLINICAL STUDIES

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It is well recognized that long term cell cultures are poor models to study human cancer, largely because of loss of clonal heterogeneity, accumulation or loss of genomic alterations and adaptation to a highly artificial environment. Patient-derived orthotopic xenografts (PDOX) based on organotypic three-dimensional tumor spheroids from human glioma samples are proposed to represent a reliable and clinically-relevant animal model. We have generated a living biobank of PDOX models from 34 glioma patients (grade III and IV), including longitudinal patient samples with matched recurrent tumors. Using an efficient orthotopic xenografting procedure we obtain an overall tumor

take-rate of close to 80%. We show that our glioma PDOX retain the genetic and epigenetic profiles of primary patient biopsies throughout several generations of xenotransplantation. In particular they not only faithfully recapitulate gene amplification and expression of EGFR and EGFRvIII variant in a reproducible manner, also amplification and expression of rarer patient-specific EGFR variants is maintained. Overall genome-wide transcriptomic profiles of PDOX remain very similar to patient biopsies and correlate better with the GBM cohort of TCGA (538 GBM samples) than conventional cell lines. Observed differences at the transcriptomic level are largely based on the replacement of human to mouse stromal cells, which impacts on the molecular sub-classification of GBM. We conclude that glioma PDOX models closely reflect patient heterogeneity and treatment response, and thus represent appropriate avatars for reproducible pre-clinical trials. Furthermore, by combining profiling of the somatic mutational landscape with large-scale drug screening, PDOX-derived tumor organoids can elucidate druggable targets and tumor response profiles in a personalized patient-specific manner.

TMOD-30. ANTI-PD-L1 ANTIBODY ENHANCES RADIATION INDUCED ABSCOPAL RESPONSE IN MURINE BRAIN TUMORS

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Immunotherapy approaches for glioblastoma multiforme have been thus far largely unsuccessful, suggesting unappreciated complexity in glioma biology and immunology. The intra-tumoral heterogeneity of these intrinsic brain tumors results in therapies killing only a subset of the tumor cells; therefore, therapeutic success will require achieving and optimizing an "abscopal effect" where tumor cells not specifically targeted are recognized and attacked as bystanders by the immune system. We have modified an immune-competent, genetically-driven mouse glioma model where a portion of the tumor burden is treated and another untreated portion is used as a readout of therapeutic efficacy. We find that following radiation of one lesion, anti-PD-L1 therapy enhances the abscopal response (macrophages and T-cells) to the un-irradiated lesion. In gliomas with few baseline T-cells, the anti-PD-L1-enhanced abscopal response occurs as an anti-PD-L1-driven, macrophage-associated, and ERK-dependent increase in phagocytosis of tumor cells. Our results indicate that combined radiation and anti-PD-L1 therapy for gliomas results in peripherally-derived macrophages being responders in tumors with few baseline T-cells in the microenvironment.

TMOD-31. NOVEL HETEROGENEOUS GLIOBLASTOMA MODELS TO OPTIMIZE HUMAN TUMORICIDAL NEURAL STEM CELL THERAPY

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Tumor-homing neural stem cell (NSC) therapy is a promising new strategy that recently entered human patient testing for glioblastoma (GBM). The success of NSCs in the clinic will depend on their ability to overcome the heterogeneity and invasiveness of patient GBM, and the development of preclinical models that recapitulate these key hurdles is essential to understanding therapeutic strengths and weaknesses. After using live organotypic brain slices to quantify the growth, migration, and drug sensitivities of different GBM lines, we generated a heterogeneous orthotopic tumor model that incorporates three GBM lines displaying significant differences in invasiveness and drug sensitivity, and exhibits spatial heterogeneity in tumor proliferation and CD31, HIF1-α, and CD133 expression. This tumor model has allowed us to begin probing how to most effectively deliver human fibroblast-derived induced NSCs (iNSCs). iNSCs expressing TRAIL or thymidine kinase were used to measure how differences in therapeutic mechanism affect TRAIL-resistant tumor regions, simultaneous solid/invasive tumor growth, and overall survival. Several treatment groups were able to significantly increase survival, depleting the solid tumor mass while decreasing HIF1-α and CD133 expression in the tumor. To further compare therapies in a second heterogeneous GBM model, two distinct tumor populations derived from the same patient tumor were combined, tested, and treated on brain slices and *in vivo*. Overall, TRAIL and TK treatment each thrived in situations where the other failed, suggesting that treatment durability and combination therapy can be further optimized in these more realistic models by tuning when and how each treatment is delivered.

TMOD-32. GENERATION OF GLIOBLASTOMA PATIENT DERIVED INTRACRANIAL XENOGRAFTS FOR PRECLINICAL STUDIES

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Glioblastoma multiforme (GBM) is the most malignant primary brain cancer affecting adults. Therapy options for GBM have remained the same for over a decade with no significant advancements. In vitro cell line models have minimal success for screening potential therapies. Many therapies that were successful in culture have failed in the clinic, likely due to the complex microenvironment in the brain, which has yet to be reproduced in any culture model. Furthermore, high passage number of cultured cells and clonal selection fail to recapitulate the molecular and genomic signatures of GBM. We have been able to successfully establish 37 patient derived xenografts (PDX) intracranially from GBM patient tissue. Tumor derived glial stem cells isolated from patient resected tissue and implanted intracranially into an immunosuppressed mouse strain. Of the 69 patient samples analyzed, we were successful in passaging three or more generations through mice 53.6% of the time. After characterization of the xenografted tumor tissue, two different tumor growth patterns emerged: highly invasive or localized. This phenotype was likely dependent on malignancy, and previous treatment of the patient from which the xenograft was derived. Physiologically, mice exhibited symptoms more quickly with each subsequent passage, particularly in the localized tumors. Development of these physiologically relevant mice, will enable therapy screenings in a microenvironment that more closely resembles GBM and may allow development of individualized patient models which can be used for simulating treatment effectors. Once established, intracranial PDX may also be useful for personalizing medicine.

TMOD-33. ESTABLISHMENT AND PRELIMINARY EVALUATION OF BEVACIZUMAB-RESISTANT GLIOMA XENOGRAFT MODELS

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High-grade gliomas characteristically exhibit aberrant neovascularization which is thought to play an important role in GBM progression. One of the main drivers of angiogenesis in GBM is vascular endothelial growth factor A (VEGF-A), which binds to VEGF receptors on endothelial cells and stimulates neovascularization and vascular permeability. For these reasons, VEGF-A mediated signaling is an attractive target for developing anti-angiogenic therapies for GBM. Clinical trials in GBM patients have shown that treatment with bevacizumab, a humanized anti-VEGF-A antibody, is associated with improvements in radiographic response, progression-free survival and quality of life. Despite these benefits, patients inevitably develop resistance and frequently fail to demonstrate significantly better overall survival. A critical issue in GBM research is to develop a better understanding of mechanisms of resistance to VEGF-targeted therapies and their clinical importance in GBM relapse. To address this question, we established two patient derived xenograft (PDX) lines from which bevacizumab-resistant sub-lines were generated by prolonged treatment over serial passages. Parental lines were generated from tumor tissue acquired at the time of tumor resection originating from newly diagnosed, untreated patients and subcutaneously engrafted in athymic mice. Tumors from initial subcutaneous parental PDXs were then serially treated with bevacizumab (5mg/kg, IP twice weekly, 5 weeks) and passed until resistance was obtained. Sensitivity to bevacizumab was reduced by 54–87% and 52–90% in subcutaneous and intracranial models respectively. Treatment with a panel of standard of care therapies are currently underway to identify differences in response rates between parental and bevacizumab-resistant PDX models. Furthermore, studies are ongoing to characterize differences in mutational profiles, gene expression signatures, and cytokine production between bevacizumab sensitive and resistant PDX lines. Results from these studies will be used to investigate novel therapeutic combinations aimed at overcoming resistance to chemotherapies and anti-angiogenic agents in GBM.

TMOD-34. IMPROVED TREATMENT OF MELANOMA BRAIN METASTASIS USING A SYNTHETIC PEPTIDE

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Individuals with brain metastasis usually await a poor prognosis. Even with the ongoing development of novel targeted therapies, a major impediment in the treatment of brain metastasis is delivering of drugs across the blood-brain/tumor barrier. Different strategies to accomplish temporary opening of the heterogenous blood-brain barrier have been described. Here, we report on a synthetic peptide, K16ApoE, which is able to open the blood-

brain barrier for improved drug-delivery in a non-permanent manner. We carried out extensive dynamic contrast enhanced magnetic resonance imaging experiments to study the ability of the peptide to open the blood-brain barrier in healthy mice. We further performed dynamic positron emission/computer tomography, with F18-labeled albumin and IgG to validate an open blood-brain barrier after co-injecting with K16ApoE. Further, we studied the effects of the peptide on cells and tissue *in vitro* and *in vivo*. The biodistribution of the peptide was studied in healthy mice by labeling I-125 to the imidazole ring of histidine in K16ApoE and tracking the peptide in plasma and tissue. We also conducted a treatment study on $BRAF^{V600E}$mutant brain metastases, where the treatment groups received K16ApoE before a BRAF inhibitor was administered, BRAF inhibitor only, K16ApoE only or vehicle. We found a peptide-induced therapeutic window at approximately 30 minutes using dynamic contrast enhanced magnetic resonance imaging. The dynamic positron emission/computer tomography showed cerebral uptake of albumin (~67 kDa) and IgG (~150 kDa) in healthy mice with a presumed intact blood-brain barrier. Further, we identified lysis of cells treated with K16ApoE in addition to in part endocytosis-mediated peptide uptake. *In vitro* blood-brain barrier modeling demonstrated endothelial and astrocyte cell layer regrowth within approximately three and 15 hours, respectively, after treatment with the peptide. We observed a significant treatment effect of co-injecting the peptide with a BRAF inhibitor on BRAF mutated brain metastases.

TMOD-35. CAN RARE SOX9-POSITIVE CELLS INCITE MYC-DRIVEN MEDULLOBLASTOMA RECURRENCE?

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Tumor recurrence is the main cause of death among children with medulloblastoma, the most frequent type of malignant pediatric brain tumors. The medulloblastoma subgroup Group 3 has the poorest survival of all four subgroups, and is associated with a high rate of tumor recurrence in children. Mechanisms behind medulloblastoma recurrence are not yet well understood. We found that the transcription factor SOX9 marks quiescent brain tumor stem cells and is suppressed by MYC overexpression in aggressive Group 3 tumors. By using our inducible Tet-OFF transgenic (GTML) mouse model for malignant MYCN-driven Group 3 tumors and human Group 3 MYC-driven patient-derived xenograft (PDX) models we identified rare SOX9-positive, slow-cycling brain tumor cells that are more resistant to standard chemotherapy. Dox treatment normally cures GTML transgenic animals that developed aggressive medulloblastoma by turning MYCN off. However, when crossing the Tet-OFF GTML model with a Tet-ON rtTA-Sox9 model we can redirect MYCN expression to the control of the Sox9 promoter - ultimately driving brain tumor recurrence from rare SOX9-positive cells with 100% penetrance. These recurrent tumors were actively disseminating from the hindbrain into the forebrain. Expression profiling shows that recurring tumors have increased levels of SOX9, are more inflammatory and have elevated levels of MGMT methyltransferase, compared to the primary tumors. Overexpressing SOX9 into Group 3 MB cells directly inhibited MYC, and decreased cell proliferation while promoting metastasis. Paired primary and recurrent human Group 3 and Group 4 tumor biopsies also showed significantly higher levels of SOX9 at recurrence. PDX models of Group 3 tumors further showed increased levels of SOX9 positivity in metastatic compartments. Our data unveils complex mechanisms by which dormant medulloblastoma cells fail to respond to standard therapy and generate tumor relapses.

TMOD-36. PRECISE INVESTIGATION OF CANCER STEM CELLS IN A MOUSE GLIOBLASTOMA MODEL

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Cancer stem cells (CSCs) have been shown to play a critical role in glioblastoma (GBM) pathogenesis. However, a precise and thorough understanding of these cells is still lacking. Here we design a novel mouse model to label, purify, and study cancer stem cells *in vivo*. Firstly we generate and characterize a new transgene to label neural stem/progenitor cells in the subventricular zone (SVZ) with GFP, and drive expression of CreERT2 and human diphtheria toxin receptor in the same cells (*CGD: nestin-CreERT2-H2BeGFP-hDTR)*. Following analysis with both bulk and single cell RNA sequencing of the SVZ tissue demonstrate its faithful expression in the stem/progenitor cell compartment. We then crossed the transgene with floxed alleles of the top three mutated tumor suppressor genes in GBM patients. This genetic configuration (CGD-M4: *CGD; $Nf1^{fl/+}$;$p53^{fl/+}$; $Pten^{fl/+}$*) efficiently promotes brain tumors initiated from wild-type neural stem/progenitor cells. Investigation of the tumors showed the CGD-GFP+ cells are quiescent *in vivo*, yet form more spheres *in vitro* than the GFP- cells. Further analysis with serial transplantation assays demonstrated the GFP+ cells are more competent to develop tumors and continuously maintain a quiescent population in the transplanted tumors. Diphtheria toxin treatment eliminates the CGD-GFP+ cells through the hDTR receptor in the transgene and greatly reduces the tumor bulk. The conventional chemotherapeutic drug, temozolomide, benefits only GFP- cell transplanted mice but not GFP+ cells. These cells are collected for RNA sequencing to identify genes associated with the CGD-GFP+ cells. Following CRISPR candidate screen reveals genes essential for the CGD-GFP+ cells. This study provides an unprecedented mouse model to study CSCs, which demonstrates their essential role in GBM initiation, recurrence, and drug resistance.

TMOD-37. IN VIVO SYNERGISTIC EFFECT OF CHECKPOINT BLOCKADE AND RADIATION THERAPY AGAINST CHORDOMAS IN A HUMANIZED MOUSE MODEL

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INTRODUCTION: Currently, there are no murine chordoma cell lines nor transgenic mouse models of chordomas, which prevents us from investigating the interaction between murine chordomas and murine immune cells. Thus, to scrutinize immunotherapy (IT) against chordomas, the development of a humanized mouse model of chordomas, where human thymus, CD34+ stem cells, and chordomas are co-transplanted to engraft human immune system into mice, is imperative. We aimed to investigate synergistic effect between IT and radiation therapy (RT) against chordomas using this model. METHODS: Fifteen 10-12-week-old NSG mice were sub-lethally irradiated and then implanted with human fetal thymic tissue and CD34+ stem cells, whose HLA-type is partially-matched with that of the U-CH1 chordoma cell line. Reconstitution of immune cells in NSG mice was confirmed eight weeks post-transplantation, and then each animal was injected with U-CH1 subcutaneously. Next, they were treated for four weeks as follows: (A) control (n=3), (B) anti-human-PD-1 antibodies (n=4), (C) RT + isotype antibodies (n=3, 8Gy x 4), (D) anti-human-PD-1 antibodies and RT (n=5). Anti-tumor activities were monitored via tumor size, flow cytometry, immunohistochemistry, and qRT-PCR. RESULTS: One week after the treatment, on the irradiated side, (D) demonstrated lowest tumor volume, highest number of human peripheral blood mononuclear cells, highest percentages of CD8+ human T cells and CD45RO+CD4+ human T cells, and lowest percentage of PD-1+CD8+ human T cells in the tumors via flow cytometry and immunohistochemistry, and highest mRNA level of IFN-gamma in the tumors via qRT-PCR, amongst the four groups with statistical significance. CONCLUSIONS: We demonstrated that this humanized mouse model could be a revolutionary platform to investigate IT against rare cancers such as chordomas, where murine equivalents are unavailable. The direct synergistic effect between IT and RT against chordoma was observed, evidenced by lowest tumor volume, highest cytotoxic T cells, and memory T cells.

TMOD-38. GMYC: A NOVEL INDUCIBLE TRANSGENIC MODEL OF GROUP 3 MEDULLOBLASTOMA

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Group 3 medulloblastoma (MB) carry the worst prognosis of all MB. The transcription factors *MYC* and *MYCN* have been suggested drivers for a

subset of these tumors, with *MYC* amplifications (17–20%) representing the most common genetic alteration in Group 3 tumors, while *MYCN* amplifications (4–6%) are less frequent. To improve current treatment options for these patients, it is of crucial importance to decipher differential features of *MYCN*- and *MYC*-driven MB and to establish accurate animal models for these patients. By driving *MYC* from the hindbrain-specific Glutamate transporter 1 (*Glt1*) promoter using a Tet-OFF system we established a novel murine model of *MYC*-driven MB (GMYC), which accurately recapitulates aggressive Group 3 MB. GMYC tumors develop without any p53 mutations and with ~70% penetrance. Tumor-prone GMYC mice can further be cured by *MYC*-depletion through dox treatment. Comparison of transcriptional profiles between GMYC and our *MYCN*-driven GTML tumors revealed that both models accurately represent Group 3 MB, while showing differential expression of key features of *MYC*- or *MYCN*-driven tumors. *CDKN2A* was identified as one of the top upregulated genes in our GTML model as compared to our GMYC model. *CDKN2A* encodes two tumor suppressors, p16INK4A and p14ARF, which are key regulators of cell cycle progression and activation of p53. Similar enhancement of *CDKN2A* was observed in *MYCN*-amplified as compared to *MYC*-amplified Group 3/4 patients. Tumor formation following partial or complete knockout of *CDKN2A* significantly increased tumor penetrance in GTML as compared to GMYC animals. Similarly, CDKN2A levels significantly correlate with poor prognosis in MYCN amplified MB patients while as compared to MYC amplified patients were CDKN2A levels are low. This suggests that MYC is regulating and suppressing CDKN2A during MB formation and further advocates that pharmacological restoration of CDKN2A would be a potential future therapy for this group of high-risk MB patients.

TMOD-39. A PLATELET-ACTIVATING FACTOR RECEPTOR ANTAGONIST DELAYS GLIOBLASTOMA GROWTH AND INVASION IN A MOUSE MODEL

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Glioblastoma Multiforme (GBM) is a diffuse malignant brain tumor with rapid growth and infiltration, high resistance to available therapies, and poor survival rate. The current standard of care only provides patients with an average survival of 12–14 months. Platelet-Activating Factor (PAF) is a strong mediator of inflammation, and its receptor, PAF-R, is over-activated in the microenvironment of different tumors as part of an enhanced neuroinflammatory response. PAF-R antagonists are beneficial in experimental ischemic stroke and counteract epileptogenesis. The purpose of this study was to determine if the PAF-R antagonist, LAU-0901, could delay GBM progression and improve survival. METHODS: U87MG cells were implanted in the right dorsal CA3 hippocampal region of BALB/c (nu/nu) mice. Intracranial tumor growth was quantified using *in vivo* bioluminescent imaging on days 10 and 25, and then every 2 weeks. Saline (vehicle, n=6) or LAU-0901 (60mg/kg/day; IP, n= 6) was administered daily from days 10 to 15 post GBM implantation. RESULTS: Tumor size before treatment on day 10 was identical in both groups. A large GBM mass was present in saline-treated mice and most of these mice died within 6 weeks; only two animals survived for the 10-week survival period. In contrast, tumor size was dramatically reduced by LAU-0901 treatment during the survival period. LAU-0901 treatment diminished tumor size by 80% on day 25, compared to saline-treated group. In addition, body weight increased by 30–45% in LAU-0901-treated mice at weeks 8 and 10. CONCLUSION: We have demonstrated that treatment with PAF-R antagonist, LAU-0901, decreased tumor growth, increased body weight and improved survival in a GBM mouse model. This novel experimental therapy can provide future avenues for GBM treatment.

ABSTRACTS FROM THE 3rd CNS ANTICANCER DRUG DISCOVERY AND DEVELOPMENT CONFERENCE

CADD-03. A VERSATILE AND MODULAR TARGETED NANOPARTICLE PLATFORM FOR DELIVERY OF COMBINATION THERAPIES TO ADULT AND PEDIATRIC CNS TUMORS

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Effective treatment of CNS tumors is limited by the presence of the blood-brain barrier (BBB) and rapid resistance to single agent therapies. To address these issues, we developed nanoparticles (NPs) that can be functionalized with ligands, such as transferrin (Tf-NPs), for delivery across the BBB to deliver multiple cargo to CNS tumors. In vitro uptake studies in U87MG and GL261 GBM cell lines demonstrate increased intracellular uptake of Tf-NPs compared to non-functionalized NPs in a time-dependent manner. Using intravital imaging through a cranial window, we show the ability of Tf-NPs to traverse the intact BBB in non-tumor bearing mice as well as achieve direct and durable tumor binding in intracranial orthotopic models of U87MG and GL261 GBM. Treatment of tumor-bearing mice with Tf-NPs loaded with temozolomide (TMZ) and the bromodomain inhibitor JQ1 leads to superior therapeutic effects with increased DNA damage and apoptosis that correlates with a 1.5- to 2-fold decrease in tumor burden and corresponding increase in survival compared to mice treated with drugs packaged in non-functionalized NPs or mice treated with equivalent free-drug dosing. Immunocompetent mice treated with Tf-NP-loaded drugs also show relative protection from the effects of systemic drug toxicity due to TMZ and JQ1. Preliminary intravital imaging further shows the ability of our NPs to target pediatric tumors such as medulloblastoma, demonstrating the preclinical potential of this nanoscale platform to deliver novel combination therapies to adult and pediatric CNS tumors.

CADD-04. NANOPARTICLE DELIVERY OF miRNAs TO INHIBIT GBM STEM CELLS

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Cancer cells arise from complementary genomic and epigenomic abnormalities that deregulate pathways that control cell proliferation and tissue homeostasis. Epigenetic modifications, involving deregulation of non-coding RNAs, are emerging as critical determinants of gene expression and essential drivers of neoplastic phenotypes. Our knowledge of how these complex epigenetic mechanisms operate in the context of cancer cell phenotype regulation remains limited. Non-coding RNAs, in particular miRNAs, are emerging as critical epigenetic regulators of cell fate and oncogenesis. MiRNAs act by selectively inhibiting gene expression primarily by targeting mRNA for degradation. Numerous miRNAs have been found to regulate tumorigenesis and cancer cell stemness by virtue of their capacity to target tumor-suppressing or tumor promoting transcripts. We recently showed that the coordinated actions of Oct4 and Sox2 induce a tumor-propagating stem-like state in GBM cells through a mechanism that involves the induction of DNMTs and down-regulation of a network of miRNAs through promoter DNA methylation. Two of the miRNAs repressed by Oct4/Sox2, miR-148a and miR-296-5p, efficiently inhibit the tumor propagating capacity of GBM stem-like, making them excellent candidates for therapeutic intervention. Options for treating high-grade brain tumors remain limited. Recent developments in nanomedicine provide new and exciting opportunities to treat and manage brain tumors. Cationic polymers are a class of biomaterials with great promise for targeted molecular therapeutics. We combined this cutting-edge technology with our newly discovered stem cell inhibiting miRNAs to develop nanoparticles to treat gliomas. We show these nano/miRs distribute throughout an established tumor *in vivo*, and more importantly, delivering these tumor-suppressing miRNAs using PBAE polymers inhibits the growth of established GBM tumor and prolongs survival in mouse models. Our findings demonstrate that identifying stem cell-inhibitory miRNAs in combination with current advances in nanomedicine will impact the development of novel therapies for treating GBM.

CADD-05. A NEW BIOANALYTICAL METHOD AND SINGLE-DOSE PHARMACOKINETICS FOR OXALOACETATE

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As reported at SNO 2017, oxaloacetate was found to increase survival in mice implanted with human glioblastoma multiforme by its pharmacologic activity as a potent "glutamate scavenger." The pharmacokinetics of oxaloacetate is not well understood in part due to a lack of bioanalytical methods. As such, a novel sensitive and specific LC–CMS/MS method was developed for the quantification of oxaloacetate in mouse plasma. The optimal chromatographic behavior was achieved using Venusil ASB Phenyl, 4.6 X 100mm, 5µ, 150°A using reversed phase chromatography. Sample preparation was done via protein precipitation with a derivatization reagent. Detection was performed by negative ion electrospray ionization in MRM mode, monitoring the transitions m/z 326.129→83.9 and m/z 320.917→152.100. The calibration curve showed good linearity within the range of 256.728 to 10269.100 ng/mL (r^2 ≥0.9940). The plasma pharmacokinetic profile of oxaloacetate was determined in healthy male BALB/c mice following oral administration at the doses of 250 and 1000 mg/kg body weight, respectively. Plasma pharmacokinetic parameters were determined by non-compartmental analysis using WinNonlin 6.3. Non-linear pharmacokinetics was demonstrated. At 250 mg/kg body weight, oxaloacetate pharmacokinetics was plasma exposure 4392.15 h*ng/mL, $T_{1/2}$ 0.2 hours, C_{max} 12063 ng/mL and mean retention time was 0.30 h. At 1000 mg/kg body weight dose the plasma exposure was 27695.99 h*ng/mL, $T_{1/2}$ 0.14 hours, C_{max} 80299 ng/

mL and the MRT was 0.26 hours. Oxaloacetate, with its rapid pharmacokinetics, may offer a safe adjunctive therapy to patients with GBM.

CADD-06. VISMODEGIB LOADED POLYOXAZOLINE (POx) MICELLES ENHANCE EFFICACY OF VISMODEGIB AND PROLONG MICE SURVIVAL, EMPHASIZE POTENTIAL OF POx MICELLES TO IMPROVE DRUG DELIVERY TO BRAIN TUMORS

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For the roughly 30% of medulloblastoma patients with SHH-subgroup tumors, drugs that disrupt SHH signaling offer the potential of improved efficacy with reduced toxicity compared to current, non-targeted treatment. The FDA-approved SMO inhibitor vismodegib, has been shown to be safe and effective for the treatment of basal cell carcinoma. In SHH-subgroup medulloblastomas, however, initial responses to vismodegib, have been followed by resistance that develops during treatment. We hypothesized that improving the delivery of the drug across the blood-brain barrier would forestall the development of resistance. We developed a novel, nanoparticle formulation of vismodegib, encapsulated in polyoxazoline micelles (POx-vismo). These nanoparticles showed high drug loading, and consistent, nanometer-scale particle size. We treated medulloblastoma bearing GFAP-cre/SmoM2 (G-Smo) mice with either POx-vismo or conventional vismodegib, administered by IP injection at postnatal days 12 and 13 (P12, P13) and then every other day. Both formulations induced an initial response. However, G-Smo mice treated with conventional vismodegib quickly progressed despite therapy. In contrast, POx-vismo-treated mice survived significantly longer than untreated mice or mice treated with conventional vismodegib. Our results show the superiority of POx-vismo and highlight the potential for POx micelles drug delivery to increase the efficacy of approved and experimental agents for brain tumor therapy. The anti-tumor effect of vismodegib, optimized in POx-vismo is significant and may, in combination with additional treatments such as radiation, lead to new treatment approaches.

CADD-09. TARGETING Nrf2 ANTIOXIDATIVE PATHWAY AS A NOVEL THERAPEUTIC STRATEGY FOR *IDH1*-MUTATED CANCERS

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BACKGROUND: Mutations in isocitrate dehydrogenase (*IDH1/2)* are the most prevalent genetic abnormalities in lower grade glioma. Neomorphic enzyme activity of mutant IDH1/2 leads to the production of oncometabolite 2-hydroxyglutarate, accompanied with disruption of redox balance through aberrant $NADP^+$/NADPH metabolism. The remarkable accumulation of reactive oxygen species (ROS) suggests distinctive metabolic stress and vulnerability in cancer cells, implying possible selective therapeutic approaches through targeting antioxitant pathways. METHODS: We investigated the ROS homeostasis in *IDH1* mutation transduced cells as well as patient derived *IDH1*-mutated brain tumor initiating cells (BTIC). The importance of antioxidant genes was confirmed through COX regression analysis from large cohort of *IDH1*-mutated lower grade glioma. Further, we investigated the involvement of Nrf2, the master transcriptional factor that regulates antioxidant enzymes in *IDH1*-mutated cells. Finally, we evaluated the therapeutic value of Nrf2 inhibitor in *IDH1*-mutated cancer *in vitro* and *in vivo*. RESULTS: We found that pathogenic *IDH1* mutation leads to substantial reprogramming in ROS homeostasis, highlighted with prompted ROS generation and compensatory up-regulation of ROS scavenging genes. The expression of key ROS scavenging genes not only establishes resistance for cancer cells, but also predicts poor disease outcome in *IDH1*-mutated lower grade glioma. Further, *IDH1*-mutated glioma develop dependency on Nrf2-governed antioxidant pathway, which plays a key role in modulating glutamate/glutathione metabolism, ROS detoxification and cancer cell survival. Pharmacologic targeting Nrf2 not only led to ROS-derived cytotoxicity in *IDH1*-mutated cells, but also selectively suppressed *IDH1*-mutated xenograft growth *in vivo*. CONCLUSION: Our findings showed that Nrf2 antioxidant pathway plays a central role in the biology of *IDH1*-mutated glioma. Targeting Nrf2 antioxidant pathway showed promising tumor suppressing effect, suggesting novel therapeutic approaches for *IDH1*-mutated malignancies.

CADD-10. 2-HYDROXYOLEIC ACID, A NOVEL MEMBRANE LIPID REGULATOR, DEMONSTRATES CLINICAL ACTIVITY IN HIGH-GRADE GLIOMA

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2-hydroxyoleic acid (2OHOA) is the first potential anti-cancer drug to act by modification of cell membrane lipid content. Through its unique mechanism, 2OHOA targets the membrane lipid composition and organization of cancer cells, altering downstream signaling cascades that promote tumor cell proliferation. Cancer cells demonstrate an abnormal membrane lipid microdomain phenotype rich in phosphatidylethanolamine (PE) and phosphatidylcholine (PC) and relatively sphingomyelin-poor when compared to non-malignant cells. Through its direct activity on sphingomyelin synthase 1, 2OHOA is able to alter the lipid membrane composition of cancer cells and restore the membrane to a normal sphingomyelin-rich phenotype. Ras is only able to propagate its signal cascade when located near the cell membrane and demonstrates an affinity for PE/PC microdomains. 2OHOA exposure results in replacement of PC and PE domains with sphingomyelin, thereby displacing Ras from the cell membrane to the cytoplasm where it is inert. Thus through normalization of the lipid membrane composition, 2OHOA is able to effectively downregulate the Ras signaling cascade, resulting in decreased tumor cell proliferation. A European phase I/IIa trial of 2OHOA in adult patients with high-grade glioma and other advanced solid tumors has recently been completed. The results are promising with five refractory malignant glioma patients demonstrating objective clinical benefit by RANO criteria for six or more months, including one glioblastoma patient with a sustained partial response (93% regression from baseline) of the primary lesion for over thirty-three months of therapy. The drug has been very well-tolerated in adult patients with minimal toxicity. A European phase IIb trial for adult patients with newly diagnosed high-grade glioma combining 2OHOA with standard therapy (radiotherapy and temozolomide) is being planned. A pediatric phase I trial of 2OHOA for children with high-grade glioma and other advanced solid tumors is due to open in the United States in 2018.

CADD-13. COMBINED INHIBITION OF NICOTINAMIDE PHOSPHORIBOSYLTRANSFERASE (NAMPT) AND POLY (ADP-RIBOSE) POLYMERASE (PARP) IMPAIRS GLIOBLASTOMA CELL GROWTH

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BACKGROUND: Glioblastoma is aggressive with poor prognosis. Nicotinamide phosphoribosyltransferase (NAMPT) is essential to maintain nicotinamide adenine dinucleotide metabolism during rapid proliferation and regulates poly (ADP-ribose) polymerase (PARP), which is crucial for DNA repair. Targeting both NAMPT and PARP may represent a treatment strategy in glioblastoma. We hypothesize that the combined inhibition of NAMPT and PARP can induce synergistic cell death in tumor, while sparing significant cytotoxicity in normal astrocytes. METHODS: NAMPT expression was determined in a group of six human glioblastoma cell lines and normal human astrocytes (NHA) by Western blotting. To analyze the cytotoxic effects of the treatments in tumor and normal cells, U251 and NHA cells were selected to receive FK866 (NAMPT inhibitor), Olaparib (PARP inhibitor), or both drugs for 72 hours prior to the cell viability test. RESULTS: Various levels of NAMPT expression were demonstrated in a group of glioblastoma cell lines and NHA, where U251 showed the strongest expression. We demonstrated a significant decrease of cell viability in U251 cells that were treated with FK866 in a dose-dependent manner. A 32% reduction of cell viability was demonstrated at a dose as low as 10 nM. However, a 28% reduction of cell viability was found in NHA at the EC_{50} concentration for U251 cells. When tumor cells were treated with a combination of FK866 and Olaparib at their EC_{50} concentrations (28nM and 406nM), 72% and 50% reductions of cell viability occurred in tumor cells and NHA, respectively. CONCLUSION: The combined treatment with FK866 and Olaparib enhances cytotoxicity in glioblastoma cells compared to the single-agent therapy, representing a promising therapeutic strategy. However, the toxic effects on NHA are concerning and warrant further investigations to determine a precise therapeutic window in preclinical models before the treatment is considered for clinical trials.

CADD-14. TARGETING THE CD200 CHECKPOINT FOR THE FIGHT AGAINST CENTRAL NERVOUS SYSTEM TUMORS

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Numerous ongoing clinical trials targeting immune checkpoints have failed to enhance survival in patients with CNS tumors. We are targeting a unique checkpoint, CD200, which controls the immune system through paired inhibitory and activation receptors. The CD200 checkpoint interferes

with tumor-immune interactions through multiple mechanisms: i) CD200 is secreted from tumors inducing an immunosuppressive environment, ii) CD200 is upregulated in tumor-associated vascular endothelial cells, creating an immunological barricade around the tumor microenvironment. We are targeting the activation receptor with a peptide ligand (CD200AR-L) activates antigen-presenting cells, enhancing dendritic cell maturation, cytokine production and antigen specific T cell activation. Treatment with CD200AR-L significantly extends survival in two murine glioma models. Directing the immune system to "fight the tumor", requires introduction of an antigen such as autologous or allogeneic tumor lysate for non-immunogenic tumors such as CNS tumors. In an ongoing pilot study treating companion dogs with high-grade glioma, patients receiving a canine specific CD200 peptide inhibitor in combination with the autologous tumor lysate vaccine increased median survival to 330 days, compared to 194 with lysate alone. Currently, 41% of the dogs are alive; the longest living dog is now 810 days post-surgery. 28% of dogs died of non-tumor related deaths. In contrast, 100% of the dogs in the tumor lysate-only group died of tumor recurrence. Furthermore, serum chemistry profiles and physical examinations showed that the peptide did not induce any systemic toxicity. Importantly, we have developed human CD200 peptide ligands that enhance cytokine secretion, dendritic cell maturation, and antigen-specific immune response. This innovative research may provide a significant breakthrough for the field of cancer immunotherapy. We have also demonstrated that the use of a CD200AR-L in a murine breast carcinoma model resulted in a significant survival benefit. In this light, CD200AR-L may be a powerful immunotherapy platform for other solid tumors.

CADD-15. TRANSLATIONAL DISCOVERY OF THERAPEUTIC TARGETS FOR GLIOBLASTOMA

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Glioblastoma is a deadly brain tumor with a median survival of 1.3 years after surgery, radiotherapy and chemotherapy. The dismal prognosis is due to the propensity of the tumor to invade adjacent normal brain. Although next generation sequencing (NGS) led to genomic landscaping of glioblastoma, there is little insight into the mechanism of neoplastic cell invasion, the detection of the invasive cells or their therapeutic targeting. To address these critical issues, we developed a murine model of invasive glioblastoma that mimics the human disease. Using a cancer stem-cell like human glioblastoma cell line in an orthotopic model, we isolated pairs of neoplastic cells growing either in the core of the tumor, or away for the core, in invasive secondary foci. A phenotypic assessment showed significantly decreased animal survival, increased cancer stemness and invasion in the invasive cells in comparison to core cells. Expression profiling of Core and Inv cells resulted in the identification of differentially expressed gene targets controlling metabolic pathways, cell-cell and cell-matrix adhesion and cell differentiation. A 300 gene library, including these targets, was designed for NGS of high grade diffuse glioma cases. In a series of 4 pediatric and adult WHO grade IV diffuse glioma autopsy cases with a total of 28 sequenced distinct primary and secondary tumor foci, as well as normal brain, we found mutations in several of the genes identified in the mouse model. Two categories of novel targets potentially relevant for therapy were confirmed and further addressed: extracellular matrix proteases and metabolic enzymes. These translational findings and the implications for the molecular diagnosis and therapy of glioblastoma will be discussed.

CADD-16. DUAL PI3K/Akt INHIBITION TO OVERCOME THE P-gp/BCRP DRUG EFFLUX SYSTEM FOR IMPROVED DRUG DELIVERY IN GLIOBLASTOMA THERAPY

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The blood-brain barrier is a primary obstacle for effective anticancer drug therapy of patients with glioblastoma multiforme (GBM). On a molecular level, failure of anticancer drug treatment is largely due to the blood-brain barrier efflux transporters P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP). P-gp and BCRP (P-gp/BCRP) work together to restrict anticancer drugs from crossing the barrier and from entering the brain to reach tumor targets. We found that PI3K/Akt regulates P-gp/BCRP in brain capillaries of the rodent and human blood-brain barrier. Our *in vivo* data show that combination treatment with LY294002 (PI3K inhibitor) and triciribine (Akt inhibitor) downregulates P-gp and BCRP protein expression and transport activity in brain capillaries. We also have evidence from brain capillaries isolated from GBM mice and GBM patients showing that GBM induces P-gp/BCRP overexpression in capillaries in the brain hemisphere that is *contralateral* to the primary tumor. These findings indicate that P-gp/BCRP overexpression in brain capillaries protects invasive tumor cells that are scattered throughout the brain from being targeted by anticancer drugs. To overcome this obstacle, we are currently developing a novel therapeutic strategy by targeting PI3K/Akt to transiently decrease P-gp/BCRP expression and activity, thus, creating a "*window-in-time*" during which anticancer drugs can enter the brain.

CADD-17. CT-179: AN INHIBITOR OF THE OLIG2 TRANSCRIPTION FACTOR WITH POTENT ANTI-TUMOUR ACTIVITY IN BRAIN CANCER

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High Grade Glioma (HGG) is incurable and has a median survival of less than 5% at five years, highlighting a desperate need for new therapeutic strategies. OLIG2 is a basic helix-loop-helix (bHLH) transcription factor that is expressed in neural progenitor cells during embryonic development where it sustains their replication-competent state and regulates their oligodendrocyte and motor neuron multi-lineage potential. In HGG, OLIG2 is re-expressed at high levels and drives an oncogenic program that leads to dysregulation of the cell cycle and subsequent gliomagenesis. This central role for OLIG2 in HGG initiation and growth, along with its low expression in normal tissues, identifies OLIG2 as a target for HGG therapy. We report the characterisation of an orally bioavailable small-molecule OLIG2 inhibitor, CT-179, the first bHLH transcription factor targeting drug developed for the treatment of cancer. The drug is well tolerated and easily penetrates the blood brain barrier, where it reduces brain tumour burden in orthotopic mouse and zebrafish avatar models. Mechanistically, CT-179 displayed nanomolar anti-proliferative activity and induced significant apoptosis mediated through disruption of the cell cycle that resulted in mitotic catastrophe at prometaphase. CT-179 showed enhanced anti-tumour activity in mouse models of HGG when used in combination with standard of care radiotherapy and temozolomide. These studies demonstrate that the pharmacological inhibition of OLIG2 is an effective treatment strategy for HGG that warrants rapid translation into the clinic.

CADD-18. MP-Pt(IV): A MAOB SENSITIVE MITOCHONDRIAL SMART BOMB FOR TREATING GLIOMA

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We have previously demonstrated that monoamine oxidase B (MAOB), highly elevated in glioma, may be used to catalytically convert uncharged methyl-tetrahydropyridine (MP-) groups into the mitochondrial targeted, cationic, methyl-pyridinium (P^+-) form. Our first generation mitochondrial 'smart-bomb', MP-MUS, used a nitrogen mustard 'warhead', and was demonstrated to attach glioma mitochondrial DNA *in vitro* and *in vivo*. The development of our lead compound into a clinically used therapeutic was hampered by the reactivity of the nitrogen mustard 'warhead' and thus the potential of off-target toxicity. Our second generation lead compound, MP-Pt(IV), replaces the nitrogen mustard with an unreactive platinum (IV) group. This new 'warhead' undergos ascorbate-linked reductive activation to give rise to *cis*-platin, DNA-crosslinking chemotherapeutic, able to attack gliomal mitochondrial DNA. MP-Pt(IV) is a very good substrate for MAOB, but not MAOA, and is converted by gliomal MAOB into the cationic, lipophilic mitochondrial targeting P^+-Pt(IV). The principle biological reductant capable of converting Pt(IV) into *cis*-platin is ascorbate. We show that gliomas have high levels of mitochondrial ascorbate, and these elevated levels of ascorbate reflect the elevation of glucose/dehydroxyascorbate transporters, GLUT1, GLUT 3, and GLUT4. Here we show that MP-Pt(IV) is highly effective chemotherapeutic, in *in vitro* as well as *in vivo* mouse intracranial mouse models of glioblastoma. *In vitro* studies show that we can potentiate the toxicity of MP-Pt(IV) by increasing mitochondrial ascorbate levels, by incubating cells with dehydroxyascorbate. In an *in vivo model*, we see that MP-Pt(IV) potentiates the classical chemotherapeutic agent temozolomide and also temozolomide based chemoradiation. Of note is the ability of MP-Pt(IV), like MP-MUS, to cause an elevation in mitochondrial, MAOB levels in treated cells. Treatment of glioma with MP-Pt(IV) creates the opposite of drug resistance, with treating cells becoming increasingly sensitive to MP-Pt(IV).

CADD-19. PAM-OBG: A MAOB-SPECIFIC PRODRUG INHIBITOR OF O^6-METHYLGUANINE DNA METHYLTRANSFERASE (MGMT) THAT SENSITIZES GMB TO BCNU/CCNU

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INTRODUCTION: MGMT gene methylation status indicate the levels of the DNA repair enzyme O^6-methylguanine DNA methyltransferase (MGMT) in glioma. MGMT repairs the DNA lesions caused by classical alkylating agents TMZ, BCNU and CCNU. Systemic inhibition of MGMT using the inhibitor O^6-methylguanine (OBG) in GBM patients, prior to chemotherapy with TMZ or BCNU has failed due to toxicity toward bone marrow. We have shown that the enzyme monoamine oxidase B (MAOB) is upregulated in GBM. We have designed, synthesized and tested *in vitro* and *in vivo*, a MAOB-specific prodrug version of OBG. The prodrug, PAM-OBG,

does not inhibit MGMT but is converted to OBG by MAOB. This prodrug should therefore only generate high levels of OBG in tissues with high levels of MAOB; i.e. Glioma, and only these tissues will become hyper-sensitive to DNA-alkylating agents. METHODS: PAM-OBG was synthesized by conventional chemistry. Enzyme kinetics, activity *in vitro* and in an *in vivo* intracranial primary GMB nude mouse model studies were performed in a manner similar to our previously reported work on MAOB prodrugs. RESULTS: PAM-OBG is a highly specific MAOB substrate, with a Km of only 200 microMolar. In tissue culture it is highly effective at inhibiting MGMT in cells with high MAOB activity, but not in cells where MAOB is inhibited. In an intracranial model of primary GBM we find that PAM-OBG is not toxic. It does however sensitize the brain tumors to the DNA-alkylating agents BCNU and CCNU. The efficacy of PAM-OBG with BCNU and CCNU, based on animal survival time, is six-fold. No increased toxicity of these chemotherapeutics is observed in off-target mouse tissues. CONCLUSION: We have developed a drug candidate prodrug to complement existing GBM therapy. We hope that PAM-OBG can be used to sensitize a patient's GBM to DNA-alkylating agents, during and following, radiotherapy.

CADD-21. PROTEASOME INHIBITION IS A TARGETED THERAPY FOR PTEN-DEFICIENT GLIOBLASTOMAS

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One of the most common genetic alterations in glioblastoma (30–40%) occurs in the *PTEN* (phosphatase and tensin homolog) tumor suppressor gene, where loss of function has been mechanistically linked to increased tumor cell invasion, and to a lack of radio- and chemo-therapy response. To identify new drug compounds that target PTEN-deficient brain tumors we performed a high throughput drug screen using patient-derived GBM spheres and found that PTEN-deficient samples were highly sensitive to proteasome inhibition. We confirmed this sensitivity in GBM spheres and iNPCs (inducible Neuronal Progenitor Cells) by genetically over-expressing or deleting PTEN, where PTEN over-expression decreased and PTEN-deletion increased sensitivity to the drug, respectively. Additionally, proteasome inhibition specifically suppressed tumor growth in mice of orthotopically engrafted human glioblastoma samples. Mechanistically, we determined that PTEN-deficient cells are more sensitive to proteasome inhibition due to an increase in protein synthesis rate and loss of autophagy activity associated with activation of the PI3K/mTOR pathway. This study reveals that proteasome inhibition is a targeted-therapeutic strategy for PTEN-deficient brain cancer.

CADD-23. PD-L1 CHECKPOINT BLOCKADE USING A SINGLE-CHAIN VARIABLE FRAGMENT TARGETING PD-L1 DELIVERED BY RETROVIRAL REPLICATING VECTOR (TOCA 521) ENHANCES ANTI-TUMOR EFFECT IN CANCER MODELS

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Conventional interventions for treating glioblastoma (GBM) patients has had limited success, with a median overall survival of 15–17 months. Recently, immune checkpoint inhibitors (CPIs) showed long-term response rates from 20–30% in some tumors, but no consistent clinical benefit with these agents has been demonstrated so far in GBM patients. We propose and provide preliminary evidence for a strategy using retroviral replicating vectors (RRV) to deliver CPI agents selectively to cancer cells that may circumvent such issues. An RRV encoding a single-chain variable fragment targeting PD-L1 (RRV-scFv-PDL1, Toca 521) binds to both mouse and human PD-L1 by competitive ELISA and competes for target occupancy with a commercially available monoclonal antibody against cell surface PD-L1. A dose-dependent bystander effect is observed with scFv PD-L1 protein expressed from RRV-scFv-PDL1 infected tumor cells showing saturated receptor binding to the cell surface PD-L1 of bystander cells when co-cultured with as low as 10% scFv PD-L1 expressing cells. In addition, the immune functional activity of scFv PD-L1 to reverse PD-1/PD-L1 mediated immune suppression was observed in a co-culture system *in vitro* and further supported by *in vivo* mouse models. Such models included a syngeneic orthotopic glioma showing that tumors infected with RRV-scFv-PDL1 conferred robust and durable immune-mediated antitumor activity superior to systemically administered anti-PD-1/anti-PD-L1 monoclonal antibodies. These results support the concept that RRV-scFv-PDL1 CPI strategy may provide an improved safety and efficacy profile compared to systemic monoclonal antibodies. The superior anti-tumor activity of RRV-scFv-PDL1 may be due to consistent high levels of scFv PD-L1 within the tumor microenvironment. This localized delivery approach with less concern for autoimmune adverse events may be therapeutically beneficial as an immuno-oncology agent either alone or in combination.

CADD-26. SMALL MOLECULE EPIGENETIC TARGETING OF METHYL-CPG BINDING PROTEIN 2 (MBD2) FOR MEDULLOBLASTOMA THERAPY

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Adhesion G-protein coupled receptors (ADGRs) are transmembrane proteins involved in cell-cell/matrix interactions. We found that the *ADGRB1* gene, which encodes Brain-specific angiogenesis inhibitor 1 (BAI1), is epigenetically silenced in human medulloblastomas through a methyl-CpG binding protein 2 (MBD2)-dependent mechanism. We revealed that BAI1 acts as a tumor suppressor by preventing Mdm2-mediated p53 polyubiquitination, and its loss substantially reduces p53 levels. We found BAI1 directly binds to mdm2 and sequesters it from p53. Reactivation of BAI1/p53 signaling axis by targeting the MBD2 pathway with a new brain-permeable MBD2 inhibitor suppressed human medulloblastoma growth in orthotopic xenograft models. Our findings highlight the importance of BAI1 loss in medulloblastoma and demonstrate that epigenetic restoration of its expression has therapeutic potential. Disruption of the BAI1/mdm2/p53 signaling axis through BAI1 silencing reveals a vulnerability in cancer, and offers an opportunity for therapeutic exploitation through epigenetic reactivation. We provide proof-of-principle that this can be achieved with a chemical scaffold targeting MBD2, and this lead molecule is actionable for translation into a first-in-class therapeutic intervention against medulloblastoma, and possibly other cancers (Zhu D *et al*, Cancer Cell, in press).

CADD-27. G-QUADRUPLEX DNA DRIVES GENOMIC INSTABILITY AND REPRESENTS A TARGETABLE MOLECULAR ABNORMALITY IN ATRX-DEFICIENT MALIGNANT GLIOMA

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Mutational inactivation of *ATRX* (a-thalassemia mental retardation X-linked) represents a defining molecular alteration in large subsets of malignant glioma. *ATRX* encodes a chromatin binding protein widely implicated in epigenetic regulation and remodeling. However, multiple studies have also associated its loss in cancer with replication stress, DNA damage, and copy number alterations (CNAs). The mechanisms by which ATRX deficiency drives this global genomic instability remain unclear. Here we report that *ATRX* inactivation in isogenic glioma model systems induces replication stress and DNA damage by promoting the formation of deleterious G-quadruplex (G4) secondary structure in DNA. Moreover, these effects are associated with the acquisition of disease-relevant CNAs over time. Prior work has linked G4s with genomic instability as well as CNAs in cancer. We then demonstrate, both *in vitro* and *in vivo*, that chemical G4 stabilization with CX-3543 (Quarfloxin) selectively enhances cell death in ATRX deficient isogenic cell lines as well as *ATRX*-mutant primary glioma stem cells derived from patients. Finally, we show that G4 stabilization synergizes with other DNA-damaging therapies, including ionizing radiation, in the ATRX-deficient context. Our findings clarify distinct mechanisms by which DNA secondary structure influences ATRX-deficient glioma pathogenesis and indicate that G4-stabilization may represent and attractive therapeutic strategy for the selective targeting of *ATRX*-mutant cancers. Opportunities for the development of radiosensitization approaches based on G4-stabilization are particularly intriguing, as ionizing radiation remains among the most effective non-surgical treatments for malignant glioma.

CADD-28. MELATONIN: TARGETING THE CELL'S POWERHOUSE TO FIGHT GLIOBLASTOMA

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Mitochondria are the key organelles that control the metabolic state of tumor cells. In particular, mitochondria regulate the Warburg effect, which is one of the main drivers of tumoral behaviour including stemness and resistance to chemotherapy. In previous studies, melatonin has shown to have oncostatic properties when used alone or in combination with chemoradiation. These anti-cancer properties are thought to be a consequence of melatonin's effects on the mitochondria. The present study examined the use of high concentrations of melatonin to boost the metabolic switch from anaerobic glycolysis to an oxidative phosphorylation (OXPHOS) state in glioblastoma (GBM) cells. Treatment of GBM cells with 3mM melatonin showed a significant decrease in viability (MTT $p < 0.001$) and proliferation (Ki67/DAPI ratio <0.001, RQ PCNA <0.001) after 48 hours. In addition, a significant rise in the reactive oxygen species production ($p < 0.001$) was

found. This phenomenon could be explained by an increase in the OXPHOS, inferred from the decrease in the lactate production ($p<0.005$) and the expression of pyruvate dehydrogenase kinase. The expression of stemness and differentiation genes was also evaluated. We found a significant increase in neural and oligodendrocytes differentiation markers ($p<0.005$ and $p<0.05$ respectively) after 7 days of treatment. Finally, we found that melatonin treatment reduced GBM migration capacity ($p<0.005$) and reduced matrix metalloproteinases expression. In summary, this study reveals that melatonin can force a metabolic switch inducing cell differentiation, eventually leading to a decrease in the malignant properties of GBM cells in vitro. Our findings highlight melatonin as a relevant therapeutic approach to target GBM by modulating cell metabolism.

CADD-30. A TREATMENT SIMULATOR FOR BRAIN CHEMOTHERAPY

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BACKGROUND: Dosing of systemically administered chemotherapies for brain tumors is generally designed without the benefit of quantitative estimates of spatial drug distribution in the brain, even though the integrity of the BBB varies spatially, and over time. METHODS: We have designed a "Drug Entry Simulator" which displays a patient's contrast enhanced MRI, a display of serum concentrations of drug over time, and an overlay of drug concentrations over time superimposed on the MRI. In addition, the area under the concentration time curve can be assessed for any region of interest. The mathematical modeling utilizes pharmacokinetic models with patient specific information on brain anatomy, BBB permeability incorporated via DCE studies and scaling hypotheses for different therapeutic agents. RESULTS: We have made and reported[1] preliminary assessments using results from clinical trials employing microdialysis or biopsies to determine the concentrations of agents within brain tumors. Simulations on data obtained from the individual patients shows 1) significant variability in spatial distribution, 2) poor penetration of the margins and 3) poor residence time of the agent in tissue. Studies of *efflux* of both small and large molecule agents infused directly into the brain have shown excellent agreement with direct measurements of their concentrations. CONCLUSIONS: Pretreatment estimates of drug entry, maximal concentration, time over effective concentration, and area under the concentration curve can help in planning systemic administration of therapeutic agents in patients with brain tumors. Future studies will address calibration issues with DCE, improvement of MR image acquisition, improved incorporation of characteristics of the drugs used, and continuing validation of the modeling approach. [1] Modeling the Entry of Systemically Administered Antineoplastic Agents into Brain Tumors, Brain Adjacent to Tumor, and Normal Brain. Stuart A. Grossman, Arati Desai, Marshall Pitz, Jaishri Blakeley, Jana Portnow, Martin Brady, Raghu Raghavan. *Journal of Clinical Oncology, SNO Proceedings,* 2008.

CADD-31. CD97 PERTUBATION BY NOVEL FUSION PROTEIN DAF-FC INHIBITS GBM INVASION AND INDUCES ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY

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INTRODUCTION: The poor prognosis of GBM reflects its capacity to supplement proliferative programs with pro-tumoral interactions with the microenvironment, including invasion and immunosuppression. There is a need to simultaneously target these processes, as they can be temporally, mutually exclusive phenotypes toggling back and forth. We have shown CD97 to be upregulated in GBM and capable of mediating each of these pro-tumoral functions in GBM by binding three ligands: decay accelerating factor (DAF/CD55), chondroitin sulfate, and integrins. To validate CD97 as a therapeutic target in glioblastoma, we partnered with an industry collaborator to produce a DAF-Fc fusion protein in a plant expression system combining DAF with IgG1 Fc to block CD97. METHODS: CD97 was targeted with siRNA or DAF-Fc. Scratch and matrigel invasion assays were used to assess migration and invasion, respectively. Antibody dependent cellular cytotoxicity (ADCC) assays used NK-92 human natural killer cells as effector cells and GBM or peripheral blood mononuclear cells (PBMCs) as target cells. Intracranial implantation of glioblastoma cells into athymic mice was followed by DAF-Fc or IgG treatment, with IgG1-Fc detected by immunohistochemistry. RESULTS: CD97 expression was elevated in GBM cells versus astrocytes and lymphocytes, and CD97 knockdown by siRNA reduced migration and invasion by 65–75% ($p<0.01$). Treatment of human and mouse GBM cells with human and mouse DAF-Fc inhibited migration and invasion by 60–80% ($p<0.001$). There was increased ADCC against GBM cells with increased DAF-Fc concentration and effector to target ratio ($p<0.001$), but negligible PBMC toxicity at the highest DAF-Fc dose. Systemically delivered DAF-Fc crossed the blood-brain barrier in orthotopic GBM models with specific tumoral uptake compared to normal brain. CONCLUSIONS: Our results validate DAF-as a GBM therapeutic with negligible off-target effects against normal astrocytes or PBMCs. Further preclinical evaluation in invasive murine models ahead of a phase I trial is underway.

CADD-32. MECHANISMS OF ENHANCED DRUG DELIVERY IN BRAIN TUMORS WITH FOCUSED ULTRASOUND-INDUCED TRANSIENT BLOOD-TUMOR BARRIER DISRUPTION

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Blood-brain/blood-tumor barriers (BBB and BTB) and interstitial transport may constitute major obstacles to the transport of therapeutics in brain tumors. In this study, we examined the impact of focused ultrasound (FUS) in combination with microbubbles on the transport of two relevant chemotherapy-based anticancer agents in HER2-positive breast cancer brain metastases at cellular resolution: doxorubicin, a non-targeted low molecular weight chemotherapeutic, and ado-trastuzumab emtansine (T-DM1), an antibody-drug conjugate. Using an orthotopic xenograft model of HER2-positive breast cancer brain metastasis and quantitative microscopy we demonstrate significant increases in the extravasation of both agents (7-fold and 2-fold for doxorubicin and T-DM1, respectively) and we provide evidence of increased drug penetration (>100μm vs. <20μm and 42 ± 7μm vs. 12 ± 4μm for doxorubicin and T-DM1, respectively) after application of FUS as compared to control (non-FUS). Integration of experimental data with physiologically based pharmacokinetic (PBPK) modeling of drug transport reveals that FUS in combination with microbubbles alleviates vascular barriers and enhances interstitial convective transport via an increase in hydraulic conductivity. Combination of experimental data and PBPK modeling suggests that FUS in combination with microbubbles increases the endothelial cell transmembrane transport and uptake. PBPK modeling indicates a selective increase in transvascular transport of doxorubicin through small vessel-wall pores size with a narrow range (Diameter: 10-50nm). Our work provides a quantitative framework for the optimization of FUS-drug combinations to maximize intratumoral drug delivery and facilitate the development of novel strategies to treat brain metastases.

CADD-33. PERSONALIZED PHARMACOGENOMICS USING GLIOMA PATIENT-DERIVED ORTHOTOPIC XENOGRAFTS (PDOXS)

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It is well recognized that long term cell cultures are poor models to study human cancer, largely because of loss of clonal heterogeneity, accumulation or loss of genomic alterations and adaptation to a highly artificial environment. Patient-derived orthotopic xenografts (PDOX) based on organotypic three-dimensional tumor spheroids from human glioma samples are proposed to represent a reliable and clinically-relevant animal model. We have generated a living biobank of PDOX models from 34 glioma patients (grade III and IV), including longitudinal patient samples with matched recurrent tumors. Using an efficient orthotopic xenografting procedure we obtain an overall tumor take-rate of close to 80%. We show that our glioma PDOX retain the genetic and epigenetic profiles of primary patient biopsies throughout several generations of xenotransplantation. In particular they not only faithfully recapitulate gene amplification and expression of EGFR and EGFRvIII variant in a reproducible manner, also amplification and expression of rarer patient-specific EGFR variants is maintained. Overall genome-wide transcriptomic profiles of PDOX remain very similar to patient biopsies and correlate better with the GBM cohort of TCGA (538 GBM samples) than conventional cell lines. Observed differences at the transcriptomic level are largely based on the replacement of human to mouse stromal cells, which impacts on the molecular sub-classification of GBM. We conclude that glioma PDOX models closely reflect patient heterogeneity and treatment response, and thus represent appropriate avatars for reproducible pre-clinical trials. Furthermore, by combining profiling of the somatic mutational landscape with large-scale drug screening, PDOX-derived tumor organoids can elucidate druggable targets and tumor response profiles in a personalized patient-specific manner.

CADD-35. THE DEVELOPMENT OF PERSONALIZED CAM AVATAR MODEL TO PREDICT CHEMOTHERAPEUTIC DRUG SENSITIVITY/ RESISTANCE OF GLIOMAS

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Malignant glial tumors are associated with a poor prognosis, presenting a short median patient survival and a very limited response to therapies. Although the first line therapy is standardized, there exists no consensus as to which second line treatment modality is better. We thus sought to demonstrate the feasibility of transforming our newly established expertise into personalized treatments for glioma patients by developing an advanced *in vivo* Avatar model developed from patient derived tumors. The typical medical Avatar system entails implantation of patient tumor samples in immunodeficient mice for subsequent test in drug efficacy. As the generation of mouse Avatars is a slow and costly approach, many cancer patients are set to have a significant disease progression before the results from the mouse model become available. We recently developed a rapid and cost-effective, pre-clinical model that is well suited for precision medicine – the *ex-ovo* chicken embryo ChorioAllantoic Membrane (CAM) assay. We will present data indicating that tumor growth occurs very rapidly in *ex ovo* CAMs, with measurable tumors obtained within a few days, as opposed to several weeks in mice. Even though tumor sizes are smaller in the CAM than in mice, the engrafting rate is higher and tumor sizes are more uniform. Implantation of glioma tissue fragments from 25 patients led to the successful establishment of CAM xenograft tumors which faithfully recapitulate the histology of the primary tumor for the majority of patients. Furthermore, we observed a significant inhibition of tumor growth in CAMs treated with first and second line chemotherapeutic drugs. This next-generation Avatar model has the potential to become an asset for personalized medicine in gliomas treatment.

CADD-42. EFFICACY OF MUTANT INTERLEUKIN-13 ALPHA-2 RECEPTOR–TARGETED LIPOSOMAL DOXORUBICIN IN THE INTRACRANIAL BRAIN TUMOR MODEL

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Glioblastoma (GBM) is the most common primary central nervous system (CNS) tumor in the United States and yet despite aggressive treatment, the median survival remains at 8 months. About 75% of human glioblastoma tumors selectively overexpress the α_2 subtype of interleukin 13 (IL-13Rα_2), considered a "decoy receptor" for GBM, allowing it to evade apoptosis normally induced by IL-13 binding. Our laboratory and others have developed targeting strategies to take advantage of IL-13Rα_2 expression on GBMs. We showed that wildtype IL-13 targeted nanoliposomes delivering doxorubicin (WT-IL13-Dox), resulted in over 5-fold reduction of orthotopic tumors, with 60% of mice surviving for >200 days compared to mice treated with unconjugated liposomes. WT-IL13, however, also binds to the shared IL-13/ IL-4 receptor α_1 subunit found in multiple organs, including the heart and lungs, suggesting potential cross-reactivity with surrounding tissues. To address off-target effects, a mutant version of IL-13 was developed known as Targeted Quadruple Mutant-13 (TQM), which display improved binding affinity to the IL-13Rα_2 receptor, and decreased affinity to the IL-13/ IL-4 receptor α_1 subunit. Twenty mice were normalized by tumor burden into two groups, one treated with WT-IL13-Dox and the other group with TQM-13-conjugated liposomal doxorubicin (TQM-13-Lip-Dox). Each group received 5mg/kg of doxorubicin in the liposomes for 4 weeks. Mice treated with TQM-targeted liposomes had slower tumor growth, smaller tumor burden, and prolonged survival (33 vs 23 days, p=0.009) than those treated with WT-IL13 liposomes. WBC counts in WT-IL13 mice suggested they were immunocompromised in comparison to TQM mice (p=0.02), which may contribute to worse survival. These findings suggest that TQM-13 bound nanoliposomes may enable the development of targeted therapy with a decreased side effect profile for delivery of chemotherapeutic agents to GBMs.

CADD-48. microRNA-34a PACKAGED IN BACTERIAL NANOCELLS OVERCOMES THERAPEUTIC RESISTANCE IN GLIOBLASTOMA

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microRNA-34a could serve as a novel therapeutic agent since it is underexpressed in Glioblastoma and modulates the expression of multiple genes in the deregulated p53, Rb and receptor tyrosine kinase networks which confer selective growth advantage and represent significant intra-tumoral heterogeneity, a major cause of therapeutic resistance. We studied the effects of microRNA-34a transfection in three primary patient-derived lines (GBM 6, GBM118 and GBM 126, respectively belonging to classical, mesenchymal and proneural subtypes), four established cell lines (T98G, U251, A172, LN229; where T98G and U251 show primary resistance to treatment while A172 and LN229 are sensitive) and two cell lines with acquired resistance to temozolomide (A172TR, LN229TR). microRNA-34a reduced proliferation and sensitized to temozolomide (Combination Index< 0.2–0.6) and radiation (dose enhancement factor 1.7–2.2) treatment, regardless of baseline treatment resistance in all studied cell lines. We identified broadly conserved microRNA-34a binding sites in the 3'UTR of multiple mRNAs in the Glioblastoma deregulated networks and genes known to confer therapeutic resistance and validated the direct downregulation of Bcl-2 protein as a major contributor to temozolomide sensitization. Nanocells (400nm diameter), termed EDV, were derived from genetically modified bacteria, provided with a bispecific antibody targeting EGFR and loaded with microRNA-34a. EDVs were injected intravenously while temozolomide was administered by oral gavage in GBM6 orthotopic mouse model. We observed significant increases in miR-34a expression, downregulation of oncogenes and reduction in tumor growth in mice treated with microRNA-34a EDV relative to control EDV(p=0.021). Further, microRNA-34a EDV significantly improved survival and synergized with temozolomide therapy [p<0.001, median survival of control EDV, microRNA-34a EDV, control EDV with temozolomide and microRNA-34a EDV with temozolomide was 44, 48, 86 and 165+ days respectively]. In conclusion, microRNA-34a EDV counteracts therapeutic resistance and intra-tumor heterogeneity.

CADD-49. IDENTIFICATION AND VALIDATION OF AZOLES AS HK2 INHIBITORS IN GLIOBLASTOMA *IN VITRO* AND *IN VIVO*

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BACKGROUND: Hexokinase I and 2 (HK1/HK2) catalyze the first committed step in glucose metabolism, ensuring a sustained glucose concentration gradient. Glioblastomas (GBMs) over-express HK2, and we have previously shown that its loss sensitizes GBM cells to treatment. In this study, we have conducted a systematic small-drug screen to identify potential HK2 inhibitors. METHODS: Pathway analysis was conducted using Gene Set Enrichment Analysis on differentially expressed genes in control and HK2 siRNA-treated samples. The top 200 up- and down-regulated genes were used to query the Connectivity Map database for potential inhibitors. 15 candidate drugs were identified and their EC_{50} was determined in glioma cell lines, glioma stem cells (GSCs), and normal human astrocytes. Dynamic metabolic flux analysis with ^{13}C-glucose labeling followed by liquid chromatography-mass spectrometry (LC-MS) was used to assess effect of candidate drugs on tumor cell glycolytic intermediates. Xenograft mice bearing glioma stem cells or U87 cells were treated with vehicle, ketoconazole, or posaconazle (25mg/kg). Mice were sacrificed when moribund and immunohistochemistry was used to assess proliferation (Ki67) and apoptosis (TUNEL assay). Mouse brain tissue drug concentration was determined using HPLC-MS/MS. RESULTS: HK2 knockdown affected glycolysis and angiogenesis. The EC_{50} of ketoconazole and posaconazole was within clinically achievable doses and below the concentration needed to affect normal human astrocytes or stem cells (<15μM). Compared to control, a significant decreased in several intermediate metabolites of glycolysis was also observed *in vitro*. Vehicle treated xenografts had a significantly shorter survival (44 ± 2 days) compared with mice treated with ketoconazole (54 ± 4 days, p<0.05) or posaconazole (56 ± 4 days, p<0.05). Both drugs led to significant reduction in proliferation and increase in apoptosis. CONCLUSION: Azoles can target genes and pathways regulated by HK2. These pre-clinical results support the value of investigating azoles as repurposed drugs in clinical trials.

CADD-50. AN OLD STORY (MGMT) IN AN EXPANDED CONTEXT (NATIONAL CANCER DATABASE)

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BACKGROUND: O^6-methylguanine-DNA methyltransferase (MGMT), a DNA repair protein, which is expressed in majority of cancers including glioblastoma multiforme (GBM), a predictive biomarker in the alkylator based treatment of gliomas. OBJECTIVE: The objective of this study is to confirm the MGMT's role as a predictive biomarker and to re-evaluate the potential MGMT, patient subset specific, prognostic role using the National

Cancer Database (NCDB). METHOD: We used the NCDB to identify GBM patients with MGMT (N=5934) registered from 2010 through 2014 in which MGMT status was detected. Hyper methylation was detected in 40.65% patients while the remaining 59.35% were unmethylated (low methylation scores). The mean, and SD age of the patients was 60.8 ± 12.7 years. The patient population included 90.6% white, 4.95% black, 4.42% of other races (Hispanic and Asians), out of these, males 57.7% and females 43.3 %. RESULTS: The incidence of GBM was 11.5% in 2010 and 34.29% in 2014. Overall survival (OS) among GBM patients was higher among methylated patients *regardless* of therapeutic intervention (p <0.001). Unadjusted mortality risk was higher in unmethylated patients (HR=1.22; 1.09–1.34). However adjusted for other demographic and therapeutic factors the hazard ratio was further increased to 1.5 (95% CI; 1.43–1.61). The other predictors of mortality were male (HR=1.10; 1.04–1.17), white (HR=1.31; 1.12–1.52), and black vs others (HR=1.22; 1.00–1.52), 5-year increase in age (HR=1.17; 1.15–1.19), no surgical resection (HR=2.00; 1.81–2.20) and no radiation treatment (HR=2.16; 2.01–2.32). Mean survival for the methylated group was 24.22 months, whereas mean survival for the unmethylated group was 17.55 months. CONCLUSION: These results confirm that MGMT promoter methylation is the single most important factor affecting survival among GBM patients and MGMT promoter methylation was an independent indicator of better prognosis for GBM patients. The presence of a methylated MGMT promoter is a predictive biomarker for response to alkylator based therapies.

CADD-53. GLIOBLASTOMA TUMORIGENESIS – LESSONS FROM REPRODUCTIVE IMMUNOLOGY

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There is no cure for glioblastoma (GBM), the most lethal primary brain tumor in adults. Despite decades of research aimed to improve outcomes, median survival is only 14–16 months. While promising discoveries in molecular genetics and immunotherapy continuously enhance our understanding of GBM and inspire new treatment approaches, challenges related to tumor-heterogeneity, inevitable recurrence, and poor patient tolerance limit treatment. The emergent need for paradigm-shifting approaches to to improve GBM-targeting is evident. Although never previously comprehensively reported, pregnancy/fetal-development shares striking similarities with gliobastomagenesis. Developing in an immune-deviated host, within an immune-privileged compartment, shielded and maintained by a proficiently adaptable microenvironment, both glioblastoma and fetal development thrive. After nine-months of pregnancy, biological and immune processes give-way to delivery (fetal-allograft rejection); however, GBM clearance is not so well orchestrated. By investigating the stages of these biologically reminiscent conditions, earlier events of glioblastomagenesis may be uncovered and potentially drive new treatment paradigms in neuro-oncology. While large cohort studies have not found a causal relationship between pregnancy and incidence of glioma, retrospective reviews of pregnancy concurrent with glioma consistently report increased aggressive glioma characteristics during the mid-late pregnancy period. These changes can in-part be ascribed to the influence of pregnancy hormones and growth factors accessing the CNS. In addition, (1) Pregnancy-mediated systemic immunosuppression and cellular re-education supporting glioma immune evasion; (2) Pregnancy hormones influence tumor-promoting cellular pathways; and (3) Fetal-maternal microchimerism allows for nesting of fetal cells within the glioma, offers local immunosuppression, and promotes glioma vascular neogenesis as quasi-stem-cells. Implementing the current knowledge of reproductive processes might offer new insight into GBM implantation and development. This abstract seeks a platform to discuss the potential to exploit overlapping concepts in reproductive immunology with that of neuro-oncology to develop clinical and biologic correlates between the two disciplines and drive innovative and paradigm-shifting multidisciplinary research.

CADD-55. LOW SERUM 25 (OH) VITAMIN D LEVEL IS ASSOCIATED WITH INCREASED RISK OF PRIMARY CNS MALIGNANCY: A RETROSPECTIVE COHORT STUDY IN A VETERAN POPULATION

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BACKGROUND: Vitamin D (VitD) deficiency has been reported to be associated with numerous malignancies, presumed to be secondary to its action through VitD receptor, immune and cell cycle regulation. OBJECTIVE: To investigate the relationship between VitD (25-hydroxyvitamin D [25(OH) D]) levels and primary brain malignancy. METHODS: We conducted a retrospective cohort study of veterans who received care through VHA from January 2000 to December 2015 and had their VitD levels measured at least once over the course of their care. Primary outcome measure was the incidence of primary brain malignancy in relation to VitD levels. Exclusion criteria included diagnoses of metastatic brain cancer, meningeal, spinal, face, neck and throat malignancies; VitD measurements in the months of November to February; exposure to ionizing radiation; and presence of genetic factors predisposing to brain tumors formation. Propensity score matching and survival analyses were conducted on the cohorts to determine and compare the incidence and hazard ratios for brain malignancy between two groups of subjects. Group 1: subjects with VitD levels higher than 20 ng/ml; and Group 2: subjects with VitD deficiency defined as ≤ 20ng/ml. Kaplan Meier curves were plotted to compare the time to events between the groups. RESULTS: After matching the two groups on several confounders (Age, Gender, BMI, Smoking Status, Alcohol Abuse, Race, Ethnicity), the incidence of primary brain malignancy was significantly higher in Group 2 (384/251,636; 154 per 100,000) in comparison with Group 1 (992/694,707; 143 per 100,000). VitD deficiency was associated with higher risk of primary brain malignancy (HR 1.129, CI [1.004 -1.269] p <0.05). Kaplan Meier analysis showed subjects with vitamin D had lower probability of survival free from primary brain malignancy that was statistically significant. CONCLUSION: Findings from this study suggests increased risk of primary brain malignancy in subjects with VitD deficiency.

CADD-56. INTRA-ARTERIAL NEO100 TRANSIENTLY OPENS THE BLOOD BRAIN BARRIER

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NEO100 is a GMP grade highly purified version of perillyl alcohol (POH), a monoterpene with cell cycle inhibitor properties. It is currently administered intranasally in Phase I/IIa IND trial in the United States for patients with recurrent GBM. Recently, we have found that NEO100 may be administered in a vitro setting, resulting in transient opening of the blood brain barrier. We have confirmed this finding in-vivo using an intracardiac injection of NEO100; intracardiac injection is used as an intra-arterial model for small rodents. Evans Blue injected intravenously after intracardiac NEO100 leads to staining throughout the whole brain. This effect is seen for up to 6 hours after injection; after that time, no Evans Blue stain is seen in the brain. We then injected a small molecule (dopamine), which normally does not cross the blood brain barrier. We were able to detect dopamine in the brain via HPLC after intracardiac injection of NEO100, followed by intravenous dopamine. Having demonstrated that non BBB permeable small molecules can cross into the brain with intra-arterial NEO100, we then tested fluorescent antibodies. Again, intracardiac injection of NEO100 followed by intravenous fluorescent antibodies allowed the antibodies to be detected in the normal brain. An in-vivo intracranial model of GL26 tumor was created. The checkpoint inhibitor antibody (anti-PD1) was used. Four groups (6 animals per group) were treated: Group 1-saline alone Group 2-NEO100 alone Group 3-anti-PD1 alone Group 4-NEO100 followed by anti-PD1. Kaplan Meier survival curves were constructed. All animals in Group 1 and Group 2 passed away within one week. Group 3 had one survivor, Group 4-all six animals survived and are still doing well. We will be translating this study into a clinical trial using interventional neuroradiology to deliver NEO100, followed by intravenous administration of anti-PD1.

CADD-57. THE EFFICACY OF THERAPY WITH ABT-414, AN EGFR-TARGETING ADC, IS POTENTIALLY ALTERED BY HETEROZYGOUS DELETION OF THE ENDOCYTIC TRAFFICKING REGULATOR RBSN

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Therapy with ABT-414 (depatux-m), a humanized monoclonal antibody drug conjugate (ADC) directed toward EGFRvIII and activated wild-type EGFR linked to MMAF, has demonstrated promising clinical activity in glioblastoma (GBM) patients with amplified EGFR and is currently being evaluated in clinical trials in first-line GBM disease settings. However, not all patients with amplified EGFR respond to ABT-414 and the reason is unclear. Cancer stem-like cells (CSLCs) have been reported to be responsible for drug and radiation resistance, and we have shown previously that internalization and endocytic trafficking of the humanized mAb bevacizumab affected the survival of CSLCs. Thus, we examined the endocytic trafficking of ABT-414 in EGFRvIII+ CSLCs, and the effect of downregulating a regulator of endocytic trafficking, RBSN (rabenosyn-5). In CSLCs treated with 75μg/ml ABT-414, a fraction of ABT-414 was trafficked to a Rab4+ fast recycling compartment (40% at 5 and 15 min), and a fraction was trafficked to the Lamp1+ lysosome (20% at 30 min and 3 hr). Similarly, in a PDX model of GBM with amplified EGFR treated with ABT-414, a fraction of ABT-414 was

trafficked to the Lamp1+ lysosome (20% at 30 min and 3 hr). Similarly, in a PDX model of GBM with amplified EGFR treated with ABT-414, a fraction of ABT-414 was detected in a Rab4+ fast recycling compartment and a fraction was detected in the Lamp1+ lysosome. Downregulation of RBSN with specific-siRNA resulted in increased targeting of ABT-414 to the Lamp1+ lysosome at 30 min and a trend-toward-an-increase at 180 min. This could potentially suggest an increased release of the drug conjugate MMAF; lysosomal trafficking is necessary for release of MMAF. Expression of RBSN in a GBM tissue microarray showed very weak/absent expression in 9% of GBM biopsies, consistent with the heterozygous deletion of RBSN in 8% of GBM tumors (TCGA database) and the corresponding significant decrease in RBSN mRNA in these tumors. These data suggest that alterations in endocytic trafficking genes, such as RBSN, could affect the efficacy of ABT-414 therapy in patients.

CADD-58. PRECLINICAL DEVELOPMENT OF miR-10b ANTAGONIST FOR THE TREATMENT OF GLIOBLASTOMA

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Glioblastoma (GBM) is the most aggressive primary brain cancer with a median survival of 15 months after diagnosis. miR-10b is highly expressed in all GBM molecular subtypes, and its expression in normal brain cells is nearly undetectable. Herein, we report the status of the preclinical development of oligonucleotide antagonists of miR-10b for the treatment of GBM. A library of 218 anti-miR-10b oligonucleotides with various lengths and chemical modifications was prepared and screened using a luciferase-based cellular miR-10b activity assay and liver slice assay (to assess potential off-target inflammatory effects). Compounds were profiled in vitro using multiple functional assays including selective inhibition of cell viability and induction of apoptosis comparing GBM cell lines and other cell lines lacking miR-10b expression. Nineteen compounds were selected for further evaluation in a xenograft mouse GBM model using human LN229 GBM cells injected intracranially. An anti-miR-10b lead compound exhibited consistent in vitro and in vivo efficacy in all screening assays. A single intratumoral injection of anti-miR-10b lead compound significantly increased median survival of tumor-bearing animals by 18%, while combination treatment with temozolomide (TMZ) extended median survival time by >120% (TMZ alone increased median survival by 27%). This anti-miR-10b lead compound exhibits favorable physiochemical properties and in vivo safety profile, which support its further development toward clinical testing. Preliminary mechanistic studies indicate that inhibition of miR-10b in GBM cell lines increased apoptosis/cell death-related and decreased proliferation-related gene expression and had synergistic inhibitory effects with TMZ on tumor cell viability.

LATE-BREAKING

LTBK-04. PHASE 1 TRIAL OF WEE1 KINASE INHIBITOR ADAVOSERTIB (AZD1775) COMBINED WITH RADIATION THERAPY FOR CHILDREN WITH NEWLY DIAGNOSED DIFFUSE INTRINSIC PONTINE GLIOMA: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP PHASE 1 PILOT CONSORTIUM (ADVL1217)

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OBJECTIVES: Children with diffuse intrinsic pontine glioma (DIPG) continue to have dismal outcomes. AZD1775 is an orally available inhibitor of Wee1 kinase, a key G2-M checkpoint regulator that has been shown to cross the blood brain barrier and has demonstrated efficacy in preclinical studies of DIPG. METHODS: AZD1775 was administered orally once daily to children with newly diagnosed DIPG, only on days of radiation therapy (30 fractions of 180cGy over 6 weeks). The protocol assessed 7 dose levels (DL) starting from 50 mg/m^2/dose and escalated up to 200 mg/m^2/dose, the maximal planned dose level. Dose escalation occurred first by the number of days on which AZD1775 was administered followed by an increase in daily dosing. The entire length of radiation therapy constituted the dose limiting toxicity (DLT) period. Correlative studies included pharmacokinetic (PK) analyses as well as determination of expression of p-CDC2, p-HH3 and γ-H2AX in peripheral blood mononuclear cells (PBMC). RESULTS: As of July 31, 2018 a total of 45 subjects were enrolled. Overall, 7 subjects (1 at each dose level) were inevaluable for DLT assessment due insufficient drug exposure. Median age for enrolled patients was 6 years (range 3–21 years) and 53% were female. The combination of AZD1775 and radiation was well tolerated with predominantly grade 1 and 2 toxicities. Only two dose limiting toxicities have occurred to date (DL 4: Grade 3 ALT elevation; DL 7: Grade 4 neutropenia). PK analysis to date shows a median (range) $T_{1/2}$ of 4.4 h (1.8–6.5 hours; N=34) and oral clearance of 41 L/h/m^2 (18–118 L/h/m^2; N=34). There was no evidence of significant accumulation. Analysis of p-CDC2, p-HH3, and γ-H2AX of the first 3 DLs showed no consistent changes in PBMCs. CONCLUSION: The combination of Wee1 kinase inhibitor AZD1775 and focal radiation therapy is well tolerated in this ongoing trial.

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