PROGRAM BOOK

25th Anniversary Meeting of the IBCN

September 29th – October 1st, 2022

at

Hospital de Sant Pau

Barcelona, Spain

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Abstract Review Committee

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Tilman Todenhöfer
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2022 IBCN Travel Award Winner:

Solomon L. Woldu, MD
University of Texas Southwestern Medical Center
Dallas, Texas, USA

"Actionable genomic landscapes from a real-world cohort of localized urothelial carcinoma patients"

Please tweet about IBCN 2022!

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Travel, Hotel and IBCN Dinner Information

Airport to Hotel: Josep Tarradellas Barcelona-El Prat Airport is 13.9 km southwest of the hotel in the city center, which amounts to a 20 min drive in a Taxi/Uber without traffic and cost €30-35. There are also bus and train options: https://www.barcelona-tourist-guide.com/en/airport/barcelona-airport-transport.html
The R2 Nord RENFE train departs from Terminal 2 approximately every 30 minutes and takes 27 minutes to Passeig de Gràcia which is just 400m (5 min walk) from the hotel.

Bus to Hospital de Sant Pau: We encourage most attendees to find their own way from their accommodation to the meeting venue. One bus will depart from Catalonia Eixample 1864 Hotel at **7:00 Friday and 7:30 Saturday** traveling to Hospital de Sant Pau.One bus will also return to the hotel at the end of the meeting on each day. The venue is a 2.5 km (30 minute) walk from the hotel, leading past La Sagrada Familia. (https://goo.gl/maps/KpardZ3Nz67PZPu77)

IBCN Dinner Friday: Dinner starts at **20:00** at "Saló dels Miralls" (Hall of Mirrors) at El Gran Teatre del Liceu. Transportation is not provided for this event – please find your own way there. This is a 1.9 km (25 min) walk from the hotel, leading along La Rambla. There is an additional fee for dinner. (https://goo.gl/maps/gLNBackKLpmDJq1WA).

THURSDAY, SEPTEMBER 29th, 2022

19:00 Welcome Dinner (*Catalonia Eixample 1864, Barcelona)*

We will have the traditional "get-together" the evening prior to the meeting. This is an informal gathering with a welcome drink at 7 pm and buffet dinner at 8 pm at the hotel.

FRIDAY, SEPTEMBER 30TH, 2022 ***(Hospital de Sant Pau)***

***Breakfast on your own in the hotel **BEFORE** meeting
***One bus departure to Hospital de Sant Pau at 700 am outside Catalonia Eixample 1864
***No free access to the museum

	Introduction	
7:30	Registration	
08:00	Welcome to IBCN Meeting	Goebell/Kamat
08:05	Welcome to Barcelona	Palou/Kogevinas

Abstract Session I		
	Topic	**Nawroth & Zuiverloon**
08:10	Single nucleus RNA-sequencing of human bladder tumors delineates intra-tumor subtype heterogeneity	Schmøkel
08:20	Whole genome sequencing of 126 early age at onset bladder cancer patients identifies novel candidate risk variants	Vermeulen
08:30	Multi-omic profiling of bladder and ureteric urothelium reveals regulatory differences pertinent to urothelial carcinoma development across the urinary tract	Mason
08:40	Predictive value of molecular subtypes and APOBEC3G for adjuvant chemotherapy in urothelial bladder cancer	Szarvas
08:50	Associations between molecular subtypes and metastatic sites in urothelial cancer	Sjödahl
09:00	Discussion	

	Bladder Cancer Epidemiology	**Kogevinas & Kiemeney**
9:10	An update on bladder cancer risk factors: what is new, which areas still need to be investigated?	Kogevinas
9:30	Lifestyle and bladder cancer prognosis: just as relevant as any adjuvant treatment?	Kiemeney
9:50	Lessons learned from validation of bladder cancer RCTs using observational data.	Richters
10:10	Discussion	

10:30	**Introduction to Breakout Groups**	**Goebell**

10:35 – 10:50 Break & Poster View

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10:50 – 12:50 Industry Meets IBCN – Breakout		
Partner	**Topic**	**Facilitator**
ImmunityBio	Synergistic activity of N-803 and BCG in NMIBC	Kamat/Reddy
Janssen	Innovation and novel approach to the treatment of NMIBC and MIBC	Bivalacqua/ Spigelman/Metekohy
PacificEdge	Biomarkers in bladder cancer detection: Cxbladder clinical experience, research and pending product release	Lotan/Sfakianos/ Aboushwareb
Photocure	Photodynamic Detection and Photodynamic Therapy in Bladder Cancer	Williams/McKee/ Young-Halvorsen
Natera	MRD testing in GU malignancies: past, present and future	Dyrskjøt/Aleshin

Lunch & Poster View 12:50 – 13:50

Industry Meets IBCN – Report from Breakout		
	Partner	**Black & Williams**
13:50	ImmunityBio	Kamat
13:55	Janssen	Bivalacqua
14:00	PacificEdge	Lotan/Sfakianos
14:05	Photocure	Williams
14:10	Natera	Dyrskjøt

IBCN Speaker		
	Topic	**Todenhöfer**
14:40	STAG2: deciphering the mechanisms of action of a novel type of tumor suppressor in bladder cancer	Francisco X. Real, CNIO, Spain
15:00	Discussion	

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Abstract Session II		
	Topic	**Kamat & Sjödahl**
15:10	Prospective study on FDG-PET/CT for On-treatment Assessment of Response to Neoadjuvant or Induction Chemotherapy in Invasive Bladder Cancer	Einerhand
15:20	Updated follow-up data and biomarker analysis of pre-operative ipilimumab and nivolumab in locoregional advanced urothelial cancer (NABUCCO)	Stockem
15:30	Propensity matched comparison of radical cystectomy with trimodality therapy for muscle invasive bladder cancer: a multi-institutional study	Zlotta
15:40	Surveillance of high-grade non-muscle-invasive bladder tumours using the Xpert® Bladder Cancer Monitor: the DaBlaCa-15 randomised clinical trial	Dreyer
15:50	uromonitor®, BTA stat®, Alere NMP22® BladderChek®, and UBC® rapid test in comparison to cytology as tumor marker for urinary bladder cancer: New results of a German prospective multicentre-study	Meisl

Poster Session with Wine & Snacks		
16:00	Short presentations of all posters at podium	Dyrskjøt

Ajdourn		
18:00	Closing words. One bus will return to hotel	Goebell/Kamat

IBCN Dinner	
20:00	"Saló dels Miralls" (Hall of Mirrors) at El Gran Teatre del Liceu *(Please find your own way to dinner. Guests must have pre-registered and paid the fee.)*

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SATURDAY, OCT 1st, 2022 *(Hospital de Sant Pau)*

***Breakfast on your own in the hotel **BEFORE** meeting
***One bus departure from hotel at 730 am
***No free access to the museum

8:00	IBCN – General Assembly	Members Only

Keynote		
	Topic	**Seiler**
9:00	Spatial prioritisation of cellular interactions in the tumor microenvironment	Moor
9:20	Discussion	

Keynote		
	Topic	**Dyrskjøt**
9:30	Targeting HLA-E and NKG2A for the treatment of BCG-unresponsive non-muscle-invasive bladder cancer	Horowitz
9:50	Discussion	

Health Break & Poster View 10:00 – 10:15

Overcoming Barriers in Data Sharing		
	Topic	**Palou & Black**
10:15	GDPR and data sharing	Vlahou
10:22	UROMOL - lessons learned	Dyrskjøt
10:29	TCGA - lessons learned	Lerner
10:36	IBCN Biobank Consortium	Lotan
10:46	Roundtable Discussion	

Abstract Session III		
	Topic	**Todenhöfer & TBA**
11:20	Genome-Wide Circulating Tumor DNA for monitoring treatment response and metastatic relapse in bladder cancer	Nordentoft
11:30	Novel clinico-genomic score predicting outcomes with platinum-based chemotherapy in patients with treatment naïve, metastatic urothelial cancer	Szabados
11:40	Validation of a GATA2 methylation and FGFR3 mutation assay to predict progression in BCG-treated high-risk non-muscle-invasive bladder cancer	Olislagers
11:50	Automated, cell-based measurements can define low-grade and high-grade noninvasive papillary urothelial carcinoma and predict time to recurrence	Berman
12:00	Proteomic Profiling of Muscle Invasive Bladder Cancer Treated with Neoadjuvant Chemotherapy	Contreras-Sanz

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Lunch & Poster View 12:10 – 13:10

Oncolytic Virus Therapy		
	Topic	**Nawroth & Bivalacqua**
13:10	Oncolytic Virotherapy	Alemany
13:30	ICAM-1-Targeted Immunotherapeutic-Coxsackievirus A21 (CVA21) as an Oncolytic Agent for Non Muscle-Invasive Bladder Cancer	Pandha
13:50	Clinical Application of CG0070 in Bladder Cancer: Lessons Learned	Li
14:10	Roundtable Discussion	

Abstract Session IV		
	Topic	**Seiler & Koti**
14:40	Epigenomic mapping identifies a super-enhancer repertoire that regulates bladder cancer cell identity through distinct transcription factor networks	Groeneveld
14:50	Tumor cell-intrinsic expression of FGFR3 drives anti-PD-1 immunotherapy resistance in a murine bladder cancer model	Sweis
15:00	Immunotherapy of bladder cancer through STING activation empowered by urease-nanomotor nanoparticle system	Jeong
15:10	ATM Loss and Therapeutic Vulnerabilities in Bladder Cancer	Mouw
15:20	NPEPPS can be targeted to overcome cisplatin resistance in patient-derived bladder cancer tumoroids	Scholtes

Awards, Wrap-Up & Closing Remarks	
15:30	Awards Presentation – Goebell/Dyrskjøt Closing Remarks – Goebell/Kamat Introduction to Montreal 2023 - Kassouf
15:45	One bus will return to hotel

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Posters		
1	Matrix metalloproteineases in blood and urine can be potential biomarkers for prediction of bladder cancer outcome	Türker
2	Identification of cisplatin resistance biomarkers for urothelial carcinoma by mass spectrometry and tissue microarray analysis	Hoffmann
3	Abnormal methylation of selected tumor supressor genes as driving factors muscle-invasive, high grade bladder cancer.	Pietrusiński
4	Stromal marker fibroblast activation protein drives outcome in T1 non-muscle invasive bladder cancer	Baekelandt
5	Assessment of predictive genomic biomarkers for response to cisplatin-based neo-adjuvant chemotherapy in bladder cancer	Van Dorp
6	Actionable genomic landscapes from a real-world cohort of localized urothelial carcinoma patients	Woldu
7	Low guideline adherence to recommended use of neoadjuvant chemotherapy in patients with non-metastatic muscle-invasive bladder cancer	van Hoogstraten
8	γδ T cells involvement in bladder cancer	Nguyen
9	Quality of life in patients with high-grade NMIBC undergoing standard versus reduced frequency of BCG Instillations: results of the EAU-RF NIMBUS-trial	van der Heijden
10	The accuracy of cystoscopy in predicting detrusor invasion in newly diagnosed bladder cancer patients	Kiemeney
11	Oncological outcomes of patients with node positive disease following neoadjuvant chemotherapy and radical cystectomy for muscle-invasive bladder cancer: A multicenter observational study of the EAU Young Academic Urologists (YAU) urothelial carcinoma working group	Marcq
12	Chemotherapy and sequential immunotherapy for locally advanced urothelial cancer: the CHASIT study	Rutten
13	The Impact of Blue Light Cystoscopy Use Among Non-Muscle Invasive Bladder Cancer Patients in an Equal Access Setting: Implications to Recurrence, Time Interval to Recurrence, and Comparable Outcomes Stratified by Race	Williams
14	Concurrent chemoradiation for muscle-invasive bladder cancer using 5-fluorouracil versus capecitabine	van Hoogstraten
15	BLCA-RegMap portal: a co-regulatory influence network view of bladder cancer heterogeneity and plasticity	Elati
16	Dataset preparation to predict response to BCG treatment for high-risk non-muscle-invasive bladder cancer from histopathological images	Khoraminia
17	Exploring relationships between FGFR3 and EGFR growth factor signalling networks in normal human urothelium to better understand luminal and basal bladder cancer	Ellison
18	Immune Cellular/Molecular Dynamics and Subtype Multiplicity of BBN-induced Bladder Tumor Development	Sundi
19	Elucidating the tumor suppressor role of STAG2 in bladder cancer	Ramal
20	Control of RB/E2F1 expression level enhances the oncolytic potency of the oncolytic virus XVir-N-31	Mantwill
21	ATM/ATR as therapeutic targets to overcome resistance in platinum-adapted bladder cancer cells	Wezel
22	Ex vivo modelling of carcinogenesis using the porcine urinary bladder (PUB) as a versatile tool for stem cell differentiation	Melzer
23	Characterization of T cells during BCG therapy for NMIBC	Brochier
24	Survey of B and Plasma Cells in Bladder Tumor Microenvironments	Sfakianos
25	Immune Microenvironment Profile of Clinically Aggressive Bladder Cancer Variants	Guo

26	Intravesical instillation of Ty21a/Vivotif: a new treatment for NMIBC patients?	Lucca
27	Bladder immune responses upon intravesical Ty21a instillations in non-muscle invasive bladder cancer patients	Derré
28	Characterization of the tumor-infiltrating immune repertoire in muscle invasive bladder cancer	Benítez
29	T Cell-to-Stroma Enrichment (TSE) score: a transcriptomic marker that predicts response to immune checkpoint inhibitors in urothelial cancer	Nakauma-González
30	Urothelial Bladder Cancer microbiota identified using tumour RNA-Seq data	Alonso
31	Integrated analysis of the bacterial microbiome, differential host gene expression and immune cell profile in the tumour microenvironment of Non-Muscle Invasive Bladder Cancer.	Pandha
32	Infiltrated luminal muscle-invasive bladder tumors have favourable outcomes with neoadjuvant chemotherapy	Reike
33	Remapping TCGA bladder cohort shows different basal immune responses and tumour heterogeneity	Ungureanu
34	Characterization of molecular stroma-rich muscle-invasive bladder cancer	Koll

Friday, Sept 30th

Oral Abstracts

Single nucleus RNA-sequencing of human bladder tumors delineates intra-tumor subtype heterogeneity

Schmøkel SS[1,2], Lindskrog SV[1,2], Nordentoft I[1], Lamy P[1], Knudsen M[1], Jensen JB[2,3], Dyrskjøt L[1,2]

[1] Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark
[2] Department of Clinical Medicine, Aarhus University, Aarhus, Denmark
[3] Department of Urology, Aarhus University Hospital, Aarhus, Denmark

Introduction: Single cell technology now makes it possible to study tumor ecosystems at single cell resolution and may improve our biological understanding of disease aggressiveness and tumor heterogeneity. Single nucleus RNA-sequencing (snRNA-seq) allows profiling of single nuclei isolated from frozen tumor tissue from patients with long-term follow-up.

Methods: We performed snRNA-seq on 48 frozen bladder tumors using an optimized DroNc-seq protocol. Nuclei were isolated using IgePal lysis buffer and the Dolomite Bio platform was used to create droplets followed by library generation and sequencing. For comparison, 44 tumors had bulk total RNA-seq available and three tumors were analyzed using 10x Chromium.

Results: We obtained 117,653 nuclei from pre-processed raw sequencing data and 59,201 nuclei remained after QC filtering (1,233 nuclei per tumor and 529 expressed genes per nuclei on average). Analysis was focused on the epithelial compartment, as it constituted 91% of all nuclei. This was confirmed by comparison to snRNA-seq data obtained using 10x Chromium. UMAP visualization and clustering of all tumors were mainly driven by patient origin indicating a high level of inter-tumor heterogeneity. To explore intra-tumor subtype heterogeneity at single nucleus level, we used the bulk classification systems (UROMOL classes of non-muscle invasive BC and the consensus classes of muscle invasive BC) to classify all epithelial nuclei from tumors having >100 epithelial nuclei and at least 40% of the classifier genes expressed. Tumors displayed varying levels of intra-tumor class heterogeneity with the dominating class at the single nucleus level constituting 36-97% of all nuclei. The dominating transcriptomic class of single nuclei was consistent with the transcriptomic class from bulk RNA-seq in 73% of the tumors. Deconvolution of cellular subpopulations within bulk tumor samples using WISP demonstrated that a higher class 2a fraction was significantly associated with worse progression-free survival.

Conclusions: Our results highlight the biological complexity of bladder tumors and underline the importance of considering the extent of intra-tumor heterogeneity in clinical management of BC patients.

Whole genome sequencing of 126 early age at onset bladder cancer patients identifies novel candidate risk variants

Vermeulen SH[1*], Sabatella M[2*], van Dijk F[2], MOTIEF consortium collaborators, Kuiper RP[2,3*], Kiemeney LALM[1*]

[1] Department for Health Evidence, Radboud university medical center, Nijmegen, The Netherlands
[2] Princess Maxima Center for Pediatric Oncology, Utrecht, The Netherlands
[3] Department of Genetics, University Medical Centre Utrecht, Utrecht.
*shared authorship

Introduction: The estimated hereditary component of urinary bladder cancer (UBC) is estimated to be up to 30%, but familial clustering is limited and high penetrant germline gene variants are predominantly found in cancer syndromes that present with a broader cancer spectrum.

Methods: As an alternative approach to study the underlying genetics of UBC, we performed whole genome sequencing on individuals with an extremely early age of UBC onset (range 12-31 years; n=126). For 54 of the individuals, we were able to include parents for sequencing which allowed us to investigate inheritance patterns and focus on de novo mutations. Rare variants (population frequency <0.01%) were prefiltered on CADD scores (>15) to enrich for damaging variants and variants were classified using PathoMAN.

Results: We identified recurrent germline missense variants in known cancer predisposition genes associated with bladder cancer (RB1, TSC1, TSC2, BAP1, and AGL), and in bladder cancer driver genes (KMT2C, ZFHX3, ERCC1, and EP300) that were predicted to be pathogenic by multiple prediction tools. In addition, (likely) pathogenic mutations were identified in multiple genes not yet associated with bladder cancer predisposition. An example is a stop gain in the centrosomal protein gene CEP152 in two patients. One of the two patients had two uncles with bladder cancer, of which one could be tested and confirmed to carry this variant as well. Furthermore, in a third patient we identified a de novo mutation in CEP192. Subsequent pathway analysis revealed the presence of damaging variants in centrosome maturation genes in 13.5% of the patients, suggesting that this pathway may play a role in early age at onset bladder cancer.

Conclusions: In conclusion, a trio-design including extremely early age at onset patients may yield new information on susceptibility genes and pathogenic pathways for bladder cancer.

Multi-omic profiling of bladder and ureteric urothelium reveals regulatory differences pertinent to urothelial carcinoma development across the urinary tract

Mason AS[1], Baker SC[1], Gawne R[1], Skinner K[1], Hinley J[1], Finn R[2], Rogerson L[3], Kattan M[3], Evans A[4], Feber A[2], Southgate J[1]

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Introduction: Urothelial carcinoma occurs throughout the urinary tract. Predominance of urothelial carcinoma of the bladder (UCB) has limited study of upper tract urothelial carcinoma (UTUC). UTUC is a rare disease with worse outcomes than UCB despite their high commonality in risk factors, mutational profiles and molecular subtypes. Whilst histologically-normal urothelium from bladder and upper tract tissues appears highly convergent, we investigated the underlying genomic architecture and transcriptomic networks to identify features which explain divergent UTUC/UCB progression.

Methods: Human bladder and ureteric urothelium was isolated from surgical samples and taken for scRNAseq or bulk DNA/RNA, or expanded and re-differentiated to a biomimetic tissue. This enabled comparison of chromatin state (ATACseq) and transcriptomic profile (bulk and single-cell RNAseq) in adult tissue, and assessment of differentiation regulatory networks. Tissue-specific profiles were used to interrogate publicly available UCB and UTUC datasets.

Results: Reflective of their convergent phenotype, differentiated and native bladder and ureteric urothelium exhibited highly similar transcriptomic and chromatin profiles. Bladder urothelium typically appeared “more” differentiated, with higher expression of luminal markers, and better resolved superficial cells by scRNAseq. Archetypal differentiation-regulating transcription factors were not different between tissues. However, germ layer differences resulted in a homeobox axis: posterior in bladder, anterior in ureter. Tumours typically retained this axis, though 10% of TCGA muscle-invasive UCBs were consistent with a ureteric origin, highlighting UTUC intraluminal seeding of cancers in the bladder. Differential homeobox usage was responsible for divergent top-level transcriptomic networks in ureter and bladder urothelium. Ureteric urothelium was predominantly driven by retinoid signalling, and bladder by WNT signalling, highlighting novel therapeutic routes.

Conclusions: Despite different embryonic origins, bladder and ureter urothelium is highly similar, now confirmed down to molecular and single-cell resolution. However, the two tissues are reliant on different top-level transcriptomic regulation and this provides opportunity for a more personalised treatment of UTUC.

Predictive value of molecular subtypes and APOBEC3G for adjuvant chemotherapy in urothelial bladder cancer

Csilla Olah[1], Henning Reis[2], Michèle J. Hoffmann[3], Fabian Mairinger[4], Saskia Ting[4], Boris Hadaschik[1], Ulrich Krafft[1], Viktor Grünwald[5], Peter Nyirady[6], Melinda Varadi[6], Balázs Győrffy[7,8], Andras Kiss[9], Eszter Szekely[9], Gottfrid Sjödahl[10], Tibor Szarvas[1,6]

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Introduction: Although targeted approaches have become available in second- and third-line settings, platinum-based chemotherapy remains the standard first-line treatment for advanced muscle-invasive bladder cancer (MIBC). Therefore, prediction of platinum resistance is of utmost clinical importance. In this study, we established a routine-compatible method for the molecular classification of MIBC samples according to the most relevant classifiers and applied this method to evaluate the impact of subtypes on survival after adjuvant chemotherapy.

Methods: This retrospective study included 191 patients with advanced MIBC (pT≥3 or pN+) who underwent radical cystectomy, with or without adjuvant chemotherapy. A 48-gene panel and classifier rule set were established to determine molecular subtypes according to TCGA, MDA, LundTax, and Consensus classifications. Additionally, 12 single platinum-predictive candidate genes were assessed. The results were correlated with patients' clinicopathological and follow-up data and were validated using independent datasets.

Results: Our final evaluation of 159 patients demonstrated better survival in the luminal groups for those who received chemotherapy compared to those who did not. In contrast, no such difference was observed in basal subtypes. The use of chemotherapy was associated with better survival in patients with high APOBEC3G expression ($p<0.002$). This association was confirmed using an independent dataset of patients who received neoadjuvant platinum therapy.

Conclusions: The proposed method robustly recapitulates the most commonly used transcriptome-based subtype classifications from paraffin-embedded tissue samples. The luminal, but not basal, molecular subtypes had the highest benefit from adjuvant platinum therapy. We identified and validated APOBEC3G as a novel predictive marker for platinum-treated patients.

Associations between molecular subtypes and metastatic sites in urothelial cancer

Sjödahl G[1*], Eriksson P[2], Höglund M[2], Holmsten K[3], Abrahamsson J[1], Bernardo C[2], Ullén A[4], Liedberg F[1]

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Introduction: The most common sites for urothelial cancer (UC) dissemination are local recurrence (after curative treatment), lymph-nodes, lung, liver, and bone. Molecular subtypes in UC show profound differences in tumor biology, reflected by distinct genomic alterations and gene expression signatures, and are associated with different T-stage distributions and chemotherapy responses. To our knowledge, no studies have systematically investigated the association between molecular subtypes and metastatic sites in UC.

Methods: We studied the 146 patients treated for advanced or metastatic UC in the Southern Sweden and the Stockholm Healthcare Regions between 2004 and 2015. Patients either had a recurrence after cystectomy and peri-operative chemotherapy (n=77) or were treated for metastatic disease with chemotherapy (n=69). Recurrence or metastasis sites (RM-sites) were classified as 'Local', 'Lymph-node', 'Lung', 'Liver', 'Bone' or 'Other'. Molecular subtypes were obtained from pre-treatment FFPE tissue by established RNA- and IHC-based Lund Taxonomy classifiers and the Consensus classifier. Associations between subtypes and RM-sites were analyzed by permutation tests and presented as raw empirical p-values. Significant associations were further studied by differential gene expression analysis in the primary tumors.

Results: Patients with a Basal/Squamous primary tumor were depleted of bone metastases (p=0.0004), while patients with Urothelial-like IHC-subtype were enriched for this RM-site (p=0.0001). Patients with Genomically Unstable (GU) subtype were depleted for lung metastases (p=0.003) but enriched for RM-site 'Other' (p=0.001). Of 7 patients with brain metastasis, 6 were of the GU IHC-subtype (p=0.001). Cases with atypical RM-site given the subtype showed molecular profiles similar to other tumors of the same subtype. Beyond subtyping, differential expression analysis revealed no clear basis for organotropism in coherent signatures or single genes.

Conclusions: Taxonomic subtypes of bladder cancer are differentially associated with bone, lung, and brain metastases. The data suggest subtype-specific mechanisms of invasion, migration, tropism, or survival in the metastatic niche. Replication in relevant model systems is the next step to identify and functionally study the underlying mechanism(s).

Prospective study on FDG-PET/CT for On-treatment Assessment of Response to Neoadjuvant or Induction Chemotherapy in Invasive Bladder Cancer

Einerhand SMH[1], Voskuilen CS[1], Fransen van de Putte EE[1], Donswijk ML[2], Bruining A[3], Van der Heijden MS[4], Mertens LS[1], Hendricksen K[1], Vegt E[2], Van Rhijn BWG[1,5]

[1] Department of Urology, [2] Department of Nuclear Medicine, [3] Department of Radiology, and [4] Department of Medical Oncology, Netherlands Cancer Institute - Antoni van Leeuwenhoek Hospital, Amsterdam, The Netherlands, [5] Department of Urology, Caritas St Josef Medical Center, University of Regensburg, Regensburg, Germany

Introduction: Neoadjuvant/induction chemotherapy (NAIC) improves survival in patients with muscle-invasive bladder carcinoma (MIBC). On-treatment response assessment aims to detect chemo-(in)sensitive tumors to continue or cease NAIC. We investigated whether FDG-PET/CT could predict response to NAIC and compared to contrast-enhanced CT.

Methods: Between 2014 and 2018 we prospectively included 83 patients with MIBC (high-risk cT2-4N0M0 or cT1-4N+M0-1a). Response to NAIC was assessed after 2-3 cycles with FDG-PET/CT (EORTC criteria) and CECT (RECIST1.1 criteria). We assessed prediction of complete pathological response (pCR; ypT0N0), complete pathological downstaging (pCD; ≤ypT1N0), and progression (inoperable tumor/ypN+/M+). The reference standard was histopathology or clinical follow-up. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy were calculated.

Results: Pathological response rates were 21% for pCR, 29% for pCD, and 10% progressed. Sensitivity, specificity, PPV, NPV and accuracy of FDG-PET/CT for prediction of pCR were 53%, 75%, 36%, 86%, and 72%. Sensitivity, specificity, PPV, NPV and accuracy of CECT for prediction of pCR were 8%, 96%, 33%, 81%, and 78%. Sensitivity, specificity, PPV, NPV and accuracy of FDG-PET/CT for prediction of pCD were 92%, 34%, 37%, 91%, and 51%. Sensitivity, specificity, PPV, NPV and accuracy of CECT for prediction of pCD were 93%, 55%, 43%, 96%, and 65%. Sensitivity, specificity, PPV, NPV and accuracy of FDG-PET/CT for prediction of progression were 21%, 96%, 71%, 74%, and 73%. Sensitivity, specificity, PPV, NPV and accuracy of CECT for prediction of progression were 5%, 98%, 50%, 67%, and 67%. Higher specificity of CECT for prediction of pCD was significant (p=0.007). In all other analyses, no significant differences between FDG-PET/CT and CECT were found.

Conclusions: Neither FDG-PET/CT nor CECT were highly accurate and response was often overestimated. Our results suggest routine FDG-PET/CT has insufficient predictive power to aid in response assessment compared to CECT and more accurate methods are needed to select patients for continued treatment with NAIC.

Updated follow-up data and biomarker analysis of pre-operative ipilimumab and nivolumab in locoregional advanced urothelial cancer (NABUCCO)

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Introduction: Patients (pts) with locoregional advanced urothelial cancer (n=54) were pre-operatively treated with ipilimumab (ipi) plus nivolumab (nivo) using different dosing regimens. Pts in cohort 1 and 2A were treated with ipi 3 mg/kg (ipi-high) and pts in cohort 2B were treated with ipi 1 mg/kg (ipi-low). A response, defined as ypT0/Tis/Ta/T1N0 for biomarker purposes, was achieved in 22/38 (58%) pts in the ipi-high cohorts and in 4/14 (29%) pts in the ipi-low cohort. Here, we present updated follow-up data and an exploratory biomarker analysis to better understand the difference between ipi-low and ipi-high. Using a multiplex PCR-based NGS assay (RaDaR), we aim to confirm pre-operative plasma ctDNA detection to predict response in cohort 2.

Methods: Somatic variants associated with response were investigated using WES on baseline tumor and whole blood DNA. Plasma ctDNA status was evaluated using the RaDaR assay. Baseline PD-L1 was determined by IHC using the 22C3 pharmDx test. Tumor immune cell infiltration was studied using multiplex immunofluorescence.

Results: In an updated survival analysis (cutoff March 2022), PFS at 1yr was numerically better in the ipi-high cohorts (85% in cohort 1 and 75% in cohort 2A) vs ipi-low (55% in cohort 2B; p=0.0880). At this early analysis, especially for cohort 2, OS at 1yr was similar in ipi-high cohorts vs the ipi-low cohort (90% in cohort 1+2A vs 85% in cohort 2B; p=0.7185) The response rate in the ipi-high cohort was 69.6% in the PD-L1 positive group vs 42.9% in the PD-L1 negative group, and 42.8% in the PD-L1 positive group vs 14.3% in the PD-L1 negative group in the ipi-low cohort. A higher TMB was observed in responders compared to non-responders (cohort 1+2A+B; p=0.00099). In cohort 1, plasma ctDNA was undetectable after pre-operative treatment in 13/14 responders and in 4/10 non-responders (p=0.0088; presented AACR 2022). Confirmation of these observations on cohort 2 are pending.

Conclusions: Pre-operative ipi+nivo in stage III UC, particularly in the ipi-high cohorts, leads to encouraging PFS. PD-L1 does not predict response in ipi-high pts. TMB can potentially evolve as biomarker for response to ipi+nivo in UC. Conclusions of pending assays will be presented at the IBCN meeting.

Propensity matched comparison of radical cystectomy with trimodality therapy for muscle invasive bladder cancer: a multi-institutional study

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Introduction: Prior randomized controlled trials comparing bladder preservation to radical cystectomy (RC) for muscle-invasive bladder cancer (MIBC) closed due to lack of accrual. None are foreseen in the near future. Thus, we aimed to compare trimodality therapy (TMT, maximal transurethral resection of bladder tumor followed by concurrent chemoradiation) to radical cystectomy (RC) in matched cohorts of patients with MIBC.

Methods: This retrospective analysis included 703 patients with MIBC clinical stage T2-T3N0M0 MIBC urothelial carcinoma of the bladder (421RC/282TMT) who would have been eligible for both RC and TMT. Patients were treated at three university centers between 2005-2017. All patients had solitary tumors <7cm, no or unilateral hydronephrosis, and no extensive carcinoma in situ. Treatment propensity scores were estimated using logistic regression and patients were matched 3:1 with replacement. Adjusted Cox models and adjusted competing risk models were used with metastasis-free survival as primary endpoint.

Results: 1,116 patients (834 RC vs 282 TMT) was included in the matched cohort. After matching, age (71.3vs71.6), cT2 stage (88vs90%), presence of hydronephrosis (12vs10%), and use of (neo)adjuvant chemotherapy (60vs65%) were similar between RC and TMT. Salvage cystectomy was performed in 38(13%) patients treated by TMT. At 5 years, metastasis-free survival (73vs78%, p=0.07) was not statistically different between RC and TMT. Outcomes for RC and TMT were not different among centers. Pathological stage in the 421 RC was pT2 in 29%, pT3-4 in 42% and 24% node positive.

Conclusions: Our multi-institutional contemporary study provides the best evidence to date demonstrating similar oncologic outcomes between RC and TMT for select MIBC patients.

Surveillance of high-grade non-muscle-invasive bladder tumours using the Xpert® Bladder Cancer Monitor: the DaBlaCa-15 randomised clinical trial

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Introduction: Non-muscle-invasive bladder cancer (NMIBC) constitutes the majority of newly diagnosed bladder tumours. Scheduled frequent surveillance is indicated for NMIBC patients because of high recurrence rate and to avoid progression to muscle invasive disease. The gold standard for surveillance is flexible cystoscopy (FC). However, FC is invasive, expensive and not always sensitive enough. The Xpert® Bladder Cancer Monitor (XBCM) is a urinary biomarker that has shown promising results as a safe replacement for FC. A previous study has shown a sensitivity of 83.3%, specificity of 75.8%, negative predictive value of 97.6% and positive predictive value of 27.8% for high-grade NMIBC specifically. However, high-level evidence from randomised clinical trials is lacking before implementation of urinary biomarkers can be considered.

Methods: A randomised clinical non-inferiority trial is currently being conducted at four Danish urological departments. Patients with previous high-grade NMIBC (Ta, T1 and CIS) are randomised 1:1 between standard follow-up surveillance with FC versus an intervention arm with follow-up surveillance consisting of XBCM test, where FC is conducted only in case of positive test and for safety reasons every 12 months. Patients in both arms are examined every three-four months for two years. Participants were allowed to receive maintenance instillation (e.g. BCG) therapy during the trial.

Results: As of April 2022 all 392 projected patients have been included. Nineteen recurrences have been detected in the standard arm, and 22 in the intervention arm. Risk difference of recurrence between the control arm and intervention was 1.3% (95% CI -6.6% to 9.3%) at 12 months. Thus, no difference in detection of NMIBC is found between the the two study arms. Furthermore, to detect an equal number of recurrences only 241 FCs were performed for the intervention arm, whereas 560 FCs were performed in the control group.

Conclusions: Preliminary results indicate that XBCM is a safe alternative to cystoscopy for surveillance of NMIBC. If this is confirmed in the remaining participants, the XBCM could replace cystoscopy in most of future follow-up visits in patients with previous HG NMIBC. Follow-up of all patients will be finished April 2024 at the latest.

uromonitor®, BTA stat®, Alere NMP22® BladderChek®, and UBC® rapid test in comparison to cytology as tumor marker for urinary bladder cancer: New results of a German prospective multicentre-study

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Introduction: BTA stat®, Alere NMP22® BladderChek®, and UBC® rapid test are urine-based rapid tests for the presence of urinary bladder cancer (BC). uromonitor® is a urine-based test measuring FGFR3, KRAS and TERT mutation. This multicentre study is the first to compare the performance of all available rapid tests with urine cytology.

Methods: 499 patients with cystoscopy-verified bladder cancer (BC), 79 patients with no evidence of disease and 221 healthy controls were enrolled in this prospective study. Urine samples were analyzed by voided urine cytology, uromonitor®, BTA stat®, Alere NMP22® BladderChek®, and UBC® Rapid test. UBC® Rapid test was assessed qualitatively and quantitatively using the point-of-care (POC) system concile® Ω100 POC reader using a cutoff of ≥10 ng/ml.

Results: Urine cytology resulted in a sensitivity of 55.9%, and a specificity of 85.7%, while the Uromonitor showed a sensitivity 49.1% and a specificity of 88.7%, respectively. NMP22® showed a sensitivity of 31.1% and a specificity of 96.7%, while BTA stat® showed a sensitivity of 74.0% and a specificity 71.1%. The qualitative and quantitative UBC® Rapid test revealed a sensitivity of 45.5% and 70.7%, with a specificity of 94.0% and 79.0%. BTA stat® and the quantitative UBC® Rapid test proved to be the best dual combination with the highest overall sensitivity (58.7%) and a specificity of 88.6%. Sensitivity increased in cytology, uromonitor®, NMP22®, BTA stat® and qualitative and quantitative UBC® Rapid test to 70.3%, 55.1%, 44.6%, 84.8%, 57.2%, and 79.7%, respect

Conclusions: BTA stat® and the quantitative UBC® rapid test showed higher sensitivity in detecting BC compared to urine cytology, but at the expense of lower specificity. A dual combination of these two tests outperforms urine cytology in terms of higher sensitivity and specificity, making them a potential alternative for the detection of BC.

Saturday, Oct 1st

Oral Abstracts

Genome-Wide Circulating Tumor DNA for monitoring treatment response and metastatic relapse in bladder cancer

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Introduction: About 45 % of patients with localized muscle-invasive bladder cancer (MIBC) treated with neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) will develop metastasis within 2 years after RC. Biomarkers for early detection of minimal residual disease (MRD) after RC are needed to enable earlier treatment initiation. Tumor-informed detection of mutations in cell-free DNA (cfDNA) has shown promising results to monitor MRD. Here we implemented and applied a whole-genome sequencing (WGS) approach to circulating tumor DNA (ctDNA) monitoring for sensitive ctDNA detection.

Methods: 67 MIBC patients undergoing NAC and RC were enrolled. cfDNA was extracted from ~1mL plasma (n=509) and procured from longitudinal plasma sampling during NAC and pre-RC (response measure), post-RC (relapse monitoring) and during immunotherapy. WGS of tumor/germline pairs (30x/20x) and plasma cfDNA (>20x) facilitating detection of genome wide genomic alterations and quantification of ctDNA using the MRDetect method.

Results: For each patient we developed a tumor-informed WGS model by integrating genome-wide mutation and copy number variation data coupled with advanced signal processing and AI-based error suppression. Patient-specific somatic variant patterns were used for detection and measuring ctDNA levels in low-input blood samples by WGS. Post-RC ctDNA analysis identified patients with recurrence with 76% sensitivity and 96% specificity and with a median lead time over radiographic imaging of 161 days. ctDNA status was associated with recurrence-free ($p<0.0001$) and overall survival ($p<0.0001$). Furthermore, ctDNA clearance during NAC was also associated with recurrence-free survival ($p=0.0089$).

Conclusions: For precision oncology, we need to develop quantitative and non-invasive methodologies for tailored treatments of individual patients and to monitor them to support clinical decision-making. The results indicate the clinical potential of personalized genome-wide mutation integration as an ultra-sensitive, non-invasive method for MRD detection and treatment response monitoring.

Novel clinico-genomic score predicting outcomes with platinum-based chemotherapy in patients with treatment naïve, metastatic urothelial cancer

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Introduction: The optimal first line regimen for treatment naïve advanced urothelial cancer (aUC) remains an area of debate. A combined clinico-genomic score correlating with outcome may help treatment selection.

Methods: The study used the de-identified Flatiron Health-Foundation Medicine Incorporation (FH-FMI) clinico-genomic database (CGDB). Data originated from ~280 US oncology centers. Patients with treatment naïve aUC received front line platinum-based chemotherapy and underwent FoundationOne® tissue sample analysis. Clinical and genomic data were analyzed and correlated with overall survival (OS). Using the Cox-LASSO method, a novel performance index was developed.

Results: 268 patients with aUC treated with front-line platinum-based chemotherapy between January 2011 and March 2020 were included. 77% (206/268) had upfront metastatic disease and 43% had a documented ECOG performance status of 0. 54% and 46% received cisplatin and carboplatin containing regimens, respectively. Patients with high APOBEC had worse OS (HR 1.43 [95% CI, 1.06–1.94]; P=0.02). FGFR3 mutations and DNA damage-repair pathway alterations were not associated with improved OS. The median OS was 8.2 months (95%CI: 6.8 – 10.0). Using machine learning methods, a performance index was developed. Low albumin, low eGFR (<50ml/min), high APOBEC and mutation in LRP1B were associated with poor survival (mOS 4.7 months [95% CI, 2.9–7.9]; P<0.001).

Conclusions: Using real-world data linked with comprehensive genomic profiling, a novel performance index was developed predicting patients with poor outcomes receiving first line platinum-based chemotherapy. Patients with LRP1B mutation have poor outcomes which requires further analysis.

Validation of a GATA2 methylation and FGFR3 mutation assay to predict progression in BCG-treated high-risk non-muscle-invasive bladder cancer

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Introduction: Non-muscle-invasive bladder cancer (NMIBC) is classified into low-, intermediate- and (very) high-risk groups according to risk stratification specified in the European Association of Urology (EAU). The recommended treatment for high-risk (HR) NMIBC is a transurethral resection of the bladder tumor followed by Bacillus Calmette–Guérin (BCG) instillations. Despite BCG treatment, ~20% of HR-NMIBC progress to secondary MIBC or metastasis. Additionally, there is an ongoing global BCG shortage. The only curative treatment after progressive disease is a delayed radical cystectomy (RC), while an early RC could have an excellent oncological outcome. Therefore, there is a need to identify patients, at the time of diagnosis, that will fail BCG treatment. We have previously shown that GATA2 methylation and FGFR3 mutation status improve precision of the EAU progression risk groups. Here, we aim to validate these findings in a large and independent BCG-treated HR-NMIBC patient cohort.

Methods: We retrospectively included primary HR-NMIBC Ta/T1 BCG-treated patients between 1999-2016 from five European hospitals. SNaPshot analysis was performed on FFPE tumor samples to identify FGFR3 mutation status. Bisulfite conversion followed by SNaPshot analysis was performed to determine GATA2 methylation status. Progression-free survival (PFS) was determined using Kaplan-Meier survival analysis and differences between groups were calculated using a log-rank test.

Results: Out of 514 included patients, 25% experienced progression during a median follow-up of 68 (IQR: 54.75) months. The risk of progression could be stratified based on GATA2 methylation and FGFR3 mutation status into a good, moderate and poor (5-year PFS: 90%, 83%, 77%; n=15%, 50%, 36%, respectively) group (p=0.034). A good prognosis was characterized by GATA2 hypomethylation and mutated FGFR3, whereas a poor outcome was associated with GATA2 hypermethylation and wild-type FGFR3.

Conclusions: We validated in a large and independent BCG-treated HR-NMIBC patient cohort that a combination of FGFR3 mutation and GATA2 methylation status identifies HR-NMIBC patients at a higher risk of progression. These patient might benefit from an early RC.

Automated, cell-based measurements can define low-grade and high-grade noninvasive papillary urothelial carcinoma and predict time to recurrence

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Introduction: Noninvasive papillary urothelial carcinoma (NPUC) places great burdens on patients and healthcare systems. Histopathologic grading for NPUC guides management, yet subjective criteria and poor reliability limit its value. Here, we develop a reproducible, quantitative, and explainable NPUC grading algorithm.

Methods: Clinical timeline data were collected for 308 NPUC patients. Histologic image analysis was performed on 641 tissue microarray (TMA) cores from transurethral resections. Features of nuclear size, shape, alignment, and mitotic rate extracted using Visiopharm software were analyzed individually and combined into regression and random forest models to establish thresholds between grades. Cox proportional hazards analysis was performed to identify prognostic histological features.

Results: Whole slide grade and stage were significantly associated with RFS. Uni- and multivariate models using histologic features differentiated low- and high-grade NPUC. Variation in nuclear area alone distinguished between grades with 82% accuracy. Complex models combining features increased accuracy to 88%. To adapt these findings to whole slide images, we used deep learning to identify hotspots that are enriched for high-grade features.

Conclusions: This work represents the first demonstration of explainable quantitative criteria for NPUC grading. By identifying mitotic index and variation in nuclear size as key metrics, the work should allow pathologists to consider grade as a continuous variable that can be tuned to important outcomes like recurrence and progression. Ongoing work will optimize the prognostic value of the NPUC grading system and translate it to whole slide images for implementation.

Proteomic Profiling of Muscle Invasive Bladder Cancer Treated with Neoadjuvant Chemotherapy

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Introduction: Neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC) is recommended for muscle invasive bladder cancer (MIBC). However only ~40% of patients show an objective response. While genomic alterations and transcriptomic classifiers have predicted response to NAC in retrospective studies, proteomic analysis of MIBC in this context is lacking. Here we profile the proteome of MIBC in the context of NAC to identify potential novel prognostic biomarkers and study the biology of NAC-resistant tumors.

Methods: Pre-NAC tissue was included from 107 MIBC patients who received NAC followed by RC. Residual tumor (≥pT1N0-3) in the RC specimen was present in 62% of patients after NAC, and was available for 55 of those patients (51%). Multiregional sampling was conducted in 37/107 pre-NAC and 15/55 post-NAC samples. Benign ureter was used as control. SP3-Clinical Tissue Proteomics (SP3-CTP) and bioinformatic analysis using formalin-fixed paraffin-embedded tissue (FFPE) were performed.

Results: We quantified 9769 proteins across all samples. Unsupervised clustering of pre-NAC tissue established four clusters with distinct survival outcomes, but no difference in pT stage after NAC: CC1, with high metabolic activity and a luminal profile; CC2, with high nuclear activity; CC3 with high immune infiltration, and basal characteristics; and CC4, with high immune infiltration and increased lipid metabolism. CC3 showed worse overall survival ($p<0.01$) and aligned with the RNA-based basal subtype. Multivariable analysis adjusting for prognostic variables identified novel favorable (MAPK9 and MTIF) and unfavorable (DVL2 and NES) biomarkers. Matched analysis of pre- and post-NAC tissue identified markers indicative of NAC resistance (AZGP1 and ORM1). Multiregional analysis showed distinct proteomic tumor profiles in selected pre- and post-NAC matched samples.

Conclusions: We described four proteomic clusters with distinct biology and survival, alongside novel prognostic biomarkers. We are validating these results by immunohistochemistry in a larger NAC cohort. A non-NAC cohort will be used to confirm the prognostic vs. predictive relevance of these findings.

Epigenomic mapping identifies a super-enhancer repertoire that regulates bladder cancer cell identity through distinct transcription factor networks

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Introduction: Muscle-invasive bladder cancer (BLCA) is an aggressive disease. Consensus BLCA transcriptomic subtypes have been proposed, with two major Luminal and Basal subgroups, presenting distinct molecular and clinical characteristics. However, how these distinct subtypes are regulated remains unclear. We hypothesized that epigenetic activation of distinct super-enhancers could drive the transcriptional programs of BLCA subtypes.

Methods: We generated RNA-seq and ChIP-seq data for active (H3K27ac) and repressive histone marks (H3K27me3, H3K9me3) in 24 bladder samples including human primary tumours (13 MIBCs and 2 Non-MIBCs), cellular models (7 bladder cancer cell lines) and patient-derived Normal Human Urothelium in proliferation (NHU, n=2). Functional knock-down and knock-out experiments of putative Luminal and Basal-associated master transcription factors were performed in BLCA cell lines.

Results: Through integrated RNA-sequencing and epigenomic profiling of histone marks in primary tumours, cancer cell lines, and normal human urothelia, we established the first integrated epigenetic map of BLCA and demonstrated the link between subtype and epigenetic control. We identified the repertoire of activated super-enhancers (SEs) and highlighted Basal, Luminal and NHU-associated SEs. We revealed the super-enhancer-regulated networks of candidate master transcription factors for Luminal and Basal subgroups including FOXA1 and ZBED2 respectively. FOXA1 CRISPR-Cas9 mutation triggered a shift from Luminal to Basal phenotype, confirming its role in Luminal identity regulation, and induced ZBED2 overexpression. In parallel, we showed that both FOXA1 and ZBED2 play concordant roles in preventing inflammatory response in cancer cells through STAT2 inhibition.

Conclusions: Our study furthers our understanding of epigenetic regulation of muscle-invasive BLCA and identifies a co-regulated network of super-enhancers and associated transcription factors, providing potential targets for the treatment of this aggressive disease.

Tumor cell-intrinsic expression of FGFR3 drives anti-PD-1 immunotherapy resistance in a murine bladder cancer model

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Introduction: Immune checkpoint blockade therapy has recently shown efficacy in treating advanced urothelial bladder cancer and anti-PD-1/PD-L1 therapy is currently the standard of care. However, most patients do not respond to these drugs and resistance mechanisms remain elusive. The non-T cell-inflamed tumor microenvironment phenotype correlates with poor prognosis and immunotherapy resistance. We previously found that activating mutations in Fibroblast Growth Factor Receptor 3 (FGFR3) were exclusive to non-T cell-inflamed bladder cancers. We investigated the impact of tumor cell-intrinsic FGFR3 activation on T cell infiltration and tumor responsiveness to anti-PD-1/PD-L1 in a murine model.

Methods: We developed a syngeneic transplantable murine bladder cancer model using the MB49 cell line engineered to express FGFR3 with either the activating G370C mutation (FGFR3-G370C), a kinase-dead mutation K508M (FGFR3-K508M), a truncating mutation resulting in a secreted receptor (FGFR3sec), or control. Mice were injected subcutaneously into flank and size was measured every 3 days along with PD-L1 therapy (BioXcell clone 10F.9G2). Tumors, draining lymph nodes, and spleens were harvested at endpoint for flow cytometry.

Results: Tumors from mice inoculated with MB49-FGFR3-G370C cells showed diminished CD8+ T cell accumulation within the tumor and were resistant to anti-PD-L1 checkpoint blockade compared to MB49 controls. To determine if FGFR3-mediated immune resistance was dependent on FGFR3 kinase activity, MB49-FGFR3-K508M tumors were evaluated and unexpectedly found to be resistant to anti-PD-L1 treatment. Tumors with a secreted FGFR3 receptor (FGFR3sec) retained responsiveness to anti-PD-L1 therapy.

Conclusions: In a murine bladder cancer model, FGFR3 activation led to relative T cell exclusion and resistance to immune checkpoint blockade. The mechanism of resistance was not dependent on receptor kinase activity. Further studies are ongoing to determine the biochemical mechanisms of FGFR3-mediated immunotherapy resistance in bladder cancer.

Immunotherapy of bladder cancer through STING activation empowered by urease-nanomotor nanoparticle system

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Introduction: Approximately 80% of bladder cancers are diagnosed as non-muscle-invasive bladder cancer which requires intravesical BCG treatment to provoke immune response for high risk patients. Stimulator of interferon genes (STING) recognizes nucleotide backbones to provoke type I interferon production to accelerate immune responses. In tumor therapy, STING agonist has been noticed by its distinguished anti-tumor effect especially in locally accessible tumors. We developed intravesical STING therapy empowered by super-enhanced drug delivery system by urease-nanomotor attached nanoparticles.

Methods: Urease-nanomotor was attached to nanocomplex composed of polydopamine (PDA). The STING agonist was incorporated into the nanomotor attached PDA. Murine bladder cancer model was established by instilling MB-49 bladder cancer cells through urethra. STING agonist incorporated nanocomplex was also delivered through bladder instillation.

Results: The mobility of urease-nanomotor PDA was empowered in urine in motion analysis and its penetration was highly enhanced in ex vivo experiments using rat bladder. Furthermore, dendritic cell activation was increased by STING agonist incorporated into nanomotor attached PDA compared with free STING agonist. In murine bladder cancer model, STING agonist incorporated into urease nanomotor-PDA provided powerful anti-tumor effect by nearly eliminating established bladder tumors. Moreover cytotoxic T cell infiltration was also significantly increased by urease nanomotor empowered STING agonist.

Conclusions: Intravesical therapy with STING agonist incorporated into urease-nanomotor PDA complex provides a powerful anti-tumor effect by inducing vigorous anti-tumor immunity.

ATM Loss and Therapeutic Vulnerabilities in Bladder Cancer

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Introduction: ATM (ataxia-telangiectasia mutated) is a kinase that plays a central role in the cellular response to DNA damage. A subset of bladder tumors harbor alterations in ATM, and several studies have shown that tumors with alterations in ATM or other DNA repair genes have increased sensitivity to DNA damaging agents and/or immunotherapeutic (anti-PD1/PD-L1) agents. However, the functional contribution of ATM alterations to these clinical associations has not been studied in detail.

Methods: We first characterize classes of ATM alterations across several large bladder cancer cohorts. We then deleted ATM in multiple mouse and human bladder cell lines and measured the impact on sensitivity to bladder cancer therapeutics in vitro and in vivo. We perform comprehensive immunogenomic profiling in ATM-intact and ATM-deleted syngeneic models. We develop and optimize an ATM immunohistochemistry (IHC) assay, apply this assay to a cohort of institutional bladder cancer cases, and correlate ATM mutation status, IHC patterns, and clinical outcomes following treatment with chemotherapy and/or immunotherapy.

Results: We find that the apparent frequency of ATM alterations in bladder cancer cohorts depends strongly on the specific criteria used to nominate alterations. Deleting ATM in bladder cell lines is sufficient to increase sensitivity to direct DNA damaging agents such as cisplatin and radiation as well as DNA repair targeted agents such as PARP and ATR inhibitors. ATM deletion confers modest changes to the immune microenvironment and anti-PD1 sensitivity in syngeneic models. ATM alteration classes are strongly associated with IHC staining patterns: tumors with truncating ATM alterations frequently exhibit loss of ATM staining by IHC whereas ATM loss by IHC is uncommon among tumors with missense or no mutations. Response to cisplatin-based chemotherapy was strongly correlated with IHC staining patterns.

Conclusions: We performed an integrated genomic and functional analyses of ATM alterations in bladder cancer. We find that ATM loss is present in a subset of tumors and is sufficient to confer sensitivity to multiple bladder cancer therapeutics in preclinical systems. ATM staining patterns by IHC vary by ATM mutation class and are associated with therapy response in clinical cohorts.

NPEPPS can be targeted to overcome cisplatin resistance in patient-derived bladder cancer tumoroids.

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Introduction: Cisplatin-based chemotherapy is the recommended neoadjuvant chemotherapy (NAC) for muscle-invasive bladder cancer (MIBC) patients. Patients without residual tumor at cystectomy, a pathological complete response (pCR), have a good 5-yr OS of 80%. However, due to NAC resistance, only 25% of patients achieve a pCR. Our aim was to overcome NAC resistance and improve the number of patients achieving pCR. Hence, we investigated NPEPPS, a key player in cisplatin resistance that we previously identified to regulate cisplatin uptake into the cell via volume regulated anion channels (VRACs).

Methods: For ex vivo validation, tumoroid cultures were generated from MIBC patients (N=8). Molecular characterization of tumor specimens and corresponding tumoroids was performed by BC-specific SNaPshot mutation analysis, copy-number aberrations analysis, immunohistochemistry (IHC), and hematoxylin & eosinophilic staining (HE). The effect of NPEPPS on cisplatin resistance was investigated using cisplatin treatment combined with the NPEPPS-inhibitor tosedostat, shRNA-mediated NPEPPS knock-down or lentiviral NPEPPS over-expression, followed by CellTiter-Glo or AlamarBlue cell viability assays.

Results: Molecular characterization of tumor and tumoroid pairs confirmed that patient-specific tumor traits were maintained in tumoroids. Post-NAC tumoroids were found to be resistant to physiological cisplatin serum concentrations. Pharmacological NPEPPS inhibition or NPEPPS depletion by shRNA-mediated knock-down resensitizes NAC-resistant tumoroids to cisplatin serum concentrations. Furthermore, NPEPPS overexpression increased cisplatin resistance in cisplatin-sensitive organoids.

Conclusions: We confirm that NPEPPS is associated with cisplatin-resistance in an ex vivo MIBC tumoroid model. These findings have potential for rapid translation into the clinic and invite trials investigating tosedostat to overcome chemoresistance.

Posters

Matrix metalloproteineases in blood and urine can be potential biomarkers for prediction of bladder cancer outcome

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Introduction: Urothelial bladder cancer (UBC) is most-frequently diagnosed at the non-muscle-invasive stage (NMIBC). However, recurrences and interventions for intermediate and high-risk NMIBC patients impact quality of life. Biomarkers for patient stratification could help to avoid unnecessary interventions whilst indicating aggressive measures when required.

Methods: In a cohort of 90 treatment-naïve UBC patients, we utilised immuno-oncology-focused multiplexed Proximity Extension Assays (PEA) to analyse plasma (n=90) and urine (n=40) samples. Public single-cell and bulk RNA-sequencing data from patient tumour tissues and murine OH-BBN-induced UBCs were also explored.

Results: According to PEA analyses, MMP7 ($p=0.028$) and CCL23 ($p=0.03$) were found in higher levels at plasma of MIBC patients compared to NMIBC. CD27 ($p=0.044$) and CD40 ($p=0.04$) were found in higher levels in the urine samples of NMIBC patients. Increased plasma levels of MMP-12 were correlated with a shorter survival (HZ=1.8, $p<0.001$, 95% CI:1.3-2.5) and was an independent prognostic marker ($p<0.001$). This finding was validated in an independent patient cohort. Single-cell transcriptomics analyses indicated tumour-infiltrating macrophages as a putative source of MMP12.

Conclusions: The local production, but systemic diffusion of MMP12, suggests that MMP12 could be an important biomarker to complement histopathology-based risk stratification. Additionally, it may represent a pharmacological target in urothelial bladder cancer.

Identification of cisplatin resistance biomarkers for urothelial carcinoma by mass spectrometry and tissue microarray analysis

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Introduction: Muscle-invasive urothelial carcinoma (MIBC) is treated with cisplatin-based chemotherapy even though it is only moderately efficient in many patients due to tumor heterogeneity and development of cisplatin resistance. To provide urothelial carcinoma (UC) patients with more personalized and efficient therapeutic options molecular biomarkers for prediction of chemotherapy response are needed. Here we report about a mass spectrometry proteomics analysis of cisplatin resistant UC cell lines (UCC) compared to naïve controls and tissue microarray analysis of identified biomarker candidates.

Methods: We performed a comparative LC-MS/MS mass spectrometry analysis of three conventional UCC (RT112, T24, J82) and their cisplatin resistant sublines (LTTs). We identified common more highly abundant proteins among the LTTs constituting candidates for new cisplatin resistance protein biomarkers. The Top 10 proteins (APOBEC3B, COTL1, GLIPR1, LANCL1, NAMPT, NRP1, PDLIM7, TOP2A, TXNDC17 and GSR) were assessed by immunohistochemical stainings on a tissue microarray (TMA) from chemo-naïve samples of 100 MIBC patients who underwent postoperative platinum-based chemotherapy. In addition, the functional involvement of APOBEC3B, NAMPT, NRP1, TOP2A and GSR in cisplatin resistance was investigated by siRNA knockdown in LTTs.

Results: Increased protein levels of PDLIM7, TOP2A and GSR were independently associated with worse overall survival in multivariate analysis. Kaplan-Meier analyses demonstrated high protein levels of PDLIM7, TOP2A, NAMPT and APOBEC3B to be significantly correlated with poor overall survival. siRNA knockdown of NAMPT, GSR and particularly of TOP2A and APOBEC3B re-sensitized cisplatin resistant LTT sublines to cisplatin treatment.

Conclusions: We identified and validated new protein biomarker candidates for cisplatin resistance in UC. Their increased protein levels contribute to poor survival of bladder cancer patients, they are functionally involved in cisplatin resistance and some are even targetable.

Abnormal methylation of selected tumor supressor genes as driving factors muscle-invasive, high grade bladder cancer.

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Introduction: The mortality among men due to bladder cancer is approximately twice as high in Poland as in other European countries. Bladder cancer is a result of multistep accumulation of genetic and epigenetic alterations and exposure to environmental factors. Aberrant methylation of CpG islands has been recognized as a potential early biomarker of carcinogenesis. Additionally, these changes affect tumor stage and grade, which may have an impact on the choice of the right treatment method.

Methods: We aimed to investigate the methylation profiles of selected tumor suppressor genes to find out whether aberrant methylation can be significantly correlated with tumor stage and/or grade. Tumor DNA from carefully selected 44 patients diagnosed with NMIBC and MIBC was analyzed. Methylation profiles were examined by MS-MLPA technique. The capillary electrophoresis method provided data for calculating the percentage of methylation of particular genes. A value above 15% was considered as a positive result.

Results: Among the analyzed genes, the most frequently hypermethylated genes were CDH13, APC and RARβ. RASSF1 methylation showed a significant association with the infiltrating stage. Hypermethylation of CDH13 (AUC = 0,75) and RASSF1 (AUC = 0,67) has been statistically significantly associated with the high-grade histology and both genes are good classifiers for this condition.

Conclusions: This study confirms the association of CpG island methylation of suppressor genes with bladder cancer and suggests its potential role in the assessment of bladder cancer aggressiveness.

Stromal marker fibroblast activation protein drives outcome in T1 non-muscle invasive bladder cancer

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Introduction: Fibroblast activation protein-α (FAP) is a transmembrane peptidase and a surrogate marker for cancer-associated fibroblasts (CAFs). FAP has been linked to worse prognosis and therapy resistance in several cancers. We hypothesised that FAP might have a prognostic biomarker potential to stratify patients with high-grade (HG) T1 non-muscle-invasive bladder cancer (NMIBC).

Methods: We selected 30 patients with HG T1 NMIBC that progressed to ≥T2 disease which were pair-matched based on CUETO progression score variables with 90 patients that did not progress. After revision a final cohort of 86 patients was retained. Slides were stained for FAP, the luminal marker GATA3 and the basal marker CK5. All HG T1 tumour regions of interest (ROIs) within each patient were annotated, analysed and scored using image analysis software.

Results: FAP expression in HG T1 ROIs was significantly higher in progressors vs. non-progressors and was prognostic for recurrence-free survival, progression-free survival, cancer-specific survival, and overall survival. FAP expression in HG T1 ROIs remained strongly prognostic for these outcomes in a bivariable model corrected for adequate BCG per FDA definition. Expression of GATA3 and CK5 did not differ between progressors vs. non-progressors, and were not prognostic for these outcomes.

Conclusions: FAP might serve as an easily applicable prognostic biomarker to risk-stratify patients with HG T1 NMIBC if these results are prospectively validated in a larger series.

Assessment of predictive genomic biomarkers for response to cisplatin-based neo-adjuvant chemotherapy in bladder cancer

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Introduction: Cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy is recommended for patients with muscle-invasive bladder cancer (MIBC). A pathologic response (ypT0/Tis/Ta/T1N0) after NAC has been associated with improved long-term survival. However, clinicians are unable to predict which patients will have a pathologic response. Somatic deleterious mutations in ERCC2, gain-of-function mutations in ERBB2, and alterations in ATM, RB1 and/or FANCC have been shown previously to correlate with pathologic response after NAC in MIBC. However, none of these biomarkers have been validated in larger independent cohorts and are consequently not used in clinical practice. The objective of this study was to validate these genomic biomarkers in an independent retrospective cohort of 165 MIBC patients who had undergone NAC and radical cystectomy.

Methods: 165 MIBC patients from five different hospitals were included. All patients were treated with at least two cycles of NAC followed by radical cystectomy. DNA was isolated from diagnostic transurethral resection material and used for DNA sequencing. Genomic alterations were inferred using population databases.

Results: Somatic deleterious mutations in ERCC2 were found in 9/68 (13%) evaluable responders and in 2/95 (2%) evaluable non-responders (P=0.009). 5-year overall survival rate was 75% (95% confidence interval (CI): 50-100%) for patients with mutations in ERCC2 and 52% (95% CI: 45%-62%) for patients without mutations in ERCC2, however this was not statistically significant (P=0.2). No correlation was observed between pathological response and alterations in ERBB2 or in ATM, RB1 and/or FANCC. In an exploratory analysis, no additional genomic alterations discriminated between responders and non-responders to NAC. No further associations were identified between the aforementioned biomarkers and pathological complete response (ypT0N0) after surgery.

Conclusions: In conclusion, we validated that deleterious mutations in ERCC2 are a genomic biomarker for pathologic response to NAC. Other previously reported genomic biomarkers were not validated.

Actionable genomic landscapes from a real-world cohort of localized urothelial carcinoma patients

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Introduction: Recent targeted therapies for advanced and metastatic urothelial cancer have generated enthusiasm, but the actionable genomic landscape of early-stage disease remains largely unknown. Here, we utilized a large, real-world cohort to investigate the incidence of a broad array of genetic alterations with potential therapeutic implications at all stages of bladder cancer.

Methods: We retrospectively analyzed de-identified NGS data from 1,562 bladder cancer patients (stages I-IV) with formalin-fixed, paraffin-embedded tumor biopsies sequenced using the Tempus|xT solid tumor assay (DNA-seq of 595-648 genes at 500x coverage; whole-exome capture RNA-seq). Tumor mutational burden was calculated and PD-L1 was assessed by immunohistochemistry. For a subset of patients with tumor-normal match sequencing (n=758), additional incidental germline alterations in 46 different genes were assessed.

Results: A total of 1,562 bladder cancer tumors were investigated: stage I-II (n=165), stage III (n=211), and stage IV (n=1,186). TMB was calculated for 1,428 tumors, and TMB-high (TMB-H; ≥10 mutations per megabase) was noted in 14% of all tumors and similar across tumor stages. PD-L1 positive expression was observed in 33% of tumors with no differences across stages. Microsatellite instability high (MSI-H) status was detected in only 2 (1.2%) stage I-II tumors and 9 (0.8%) stage IV tumors. Alterations—single nucleotide variants, insertions/deletions, and copy number variants—in FGFR2/3, homologous recombination repair genes (18 genes including BRCA1/2 and ATM), additional DNA repair gene mutations (ERCC2, RB1, FANCC) and NTRK fusions were detected at similar frequencies across disease stages. In 915 patients with tumor/normal matched sequencing, we identified a low rate of incidental germline mutations in all tumors (5.2%) and in specific genes: MUTYH (1.9%), BRCA2 (0.5%), and ATM (0.8%).

Conclusions: Important subsets of patients demonstrate genetic alterations in potentially actionable molecular pathways in all stages. This analysis showed that were is minimal variability in these alterations across stages, providing rationale for early identification of genetic alterations and personalization of therapies at all stages for patients with bladder cancer.

Low guideline adherence to recommended use of neoadjuvant chemotherapy in patients with non-metastatic muscle-invasive bladder cancer

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Introduction: Although recommended in international guidelines, the survival benefit of neoadjuvant chemotherapy (NAC) in muscle-invasive bladder cancer (MIBC) remains controversial. As a result, practice variation is expected. We evaluated guideline adherence and assessed practice variation between hospitals in NAC use and its effect on survival.

Methods: In this nationwide study based on the Netherlands Cancer Registry, we identified 1,025 patients newly diagnosed with non-metastatic MIBC between November 2017 and November 2019 who underwent radical cystectomy. Patients with ECOG performance status 0-1 and creatinine clearance ≥50 mL/min/1.73m2 were considered NAC-eligible. Interhospital variation was assessed using case-mix adjusted multilevel analysis. The association between hospital-specific probabilities of NAC and survival was evaluated using a Cox proportional hazards model. All analyses were stratified by disease stage (cT2 versus cT3-4a).

Results: In total, 809 patients were considered NAC-eligible, but only 277 (34%) received NAC. Guideline adherence for NAC in cT2 was lower compared to T3-T4a disease (26% versus 55%) and interhospital variation was larger (7-57% versus 31-62%). For cT2-disease, adjusted two-year OS seems in favor of patients treated in hospitals with higher probability to administer NAC (HR 0.59, 95%CI 0.33-1.05) although not statistically significant. For cT3-4a, no significant effect was observed (HR 0.71, 95%CI 0.25-2.04).

Conclusions: Guideline adherence regarding NAC use is low and interhospital variation is large, especially for patients with cT2-disease. Our data suggest that hospitals more likely to give NAC in T2-disease perform slightly better concerning survival, although results should be interpreted with care as this was not statistically significant. Further research is warranted to elucidate the underlying mechanism. As literature is clear concerning the potential survival benefit of NAC in patients with cT3-4a disease, our data shows that guideline adherence could be improved.

γδ T cells involvement in bladder cancer

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Introduction: Bladder cancer (BCa) is a public health concern due to its prevalence and high risk of recurrence. Although Bacillus Calmette-Guérin (BCG) instillation is the gold-standard treatment for urothelial carcinoma, such treatment is associated with significant side-effects and failure, underlying the necessity for alternative immunotherapy. Tumor-infiltrating lymphocytes (TIL) are a crucial component for controlling tumor growth. Among TIL, γδ T cells sparked interest due to their potent anti-tumor functions. Notably, mouse xenograft models demonstrated the relevance of using γδ T cells as a novel therapy for BCa, but the contribution of γδ T cells in BCa patients remains unaddressed.

Methods: Therefore, we determined the proportion of intratumor γδ T cells in muscle-invasive BCa patients from The Cancer Genome Atlas (TCGA) and the frequency of blood Vδ1, Vδ2, and total γδ T cells, by flow cytometry, from 40 non-muscle and 40 muscle-invasive BCa patients, as well as 20 non-tumor patients. Finally, we investigated in vitro whether treatment could promote BCa tumor cell recognition by Vδ2 T cells.

Results: We found, in the TGCA analysis, a decrease of γδ T cells in the tumor compared to normal adjacent tissue. Yet, a high intratumor γδ T-cell proportion were associated with improved survival. In the blood of BCa patients, we also observed a lower frequency of total γδ, Vδ1 and Vδ2 T cells compared to non-tumor patients. Similarly to the TCGA analysis, a favorable clinical outcome is associated with a high frequency of γδ T cells, which may be mainly attributed to the Vδ2 T-cell subset. Furthermore, in vitro assays revealed that either BCG, Zoledronate, or anti-BTN3 agonistic antibody treatment of bladder tumor cells induced Vδ2 T-cell cytolytic and cytokine production. Strikingly, combining BCG and Zoledronate treatments elicited the most potent response by increasing the frequency and the polyfunctionality of bladder tumor-reactive Vδ2 T cells.

Conclusions: Overall, our results suggest that Vδ2 T cells may play a prominent role in bladder tumor control, and non-muscle invasive BCa patients undergoing BCG therapy may benefit from Zoledronate administration by boosting Vδ2 T cells anti-tumor activity.

Quality of life in patients with high-grade NMIBC undergoing standard versus reduced frequency of BCG Instillations: results of the EAU-RF NIMBUS-trial

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Introduction: Intravesical Bacillus Calmette-Guérin (BCG) instillations are recommended to reduce the risk of recurrence and progression in patients with high-grade non-muscle-invasive bladder cancer (NMIBC). Unfortunately, toxicity is significant during the long-term administration of BCG, often leading to treatment discontinuation. The NIMBUS trial showed a reduced number of standard-dose BCG instillations to be inferior to the standard number and dose concerning the risk of recurrence. This study evaluated whether patients in the reduced BCG instillation arm of the trial had a better Quality of Life (QoL) than patients in the standard frequency arm.

Methods: A total of 359 eligible patients from 51 study sites across Europe were randomized in either of the two treatment arms between December 2013 and July 2019. The standard frequency arm (n=182) consisted of 6 weeks of induction followed by 3 weeks of maintenance at months 3, 6, and 12 (15 instillations). The reduced frequency arm (n=177) consisted of induction at weeks 1, 2, and 6 followed by maintenance instillations at weeks 1 and 3 of months 3, 6, and 12 (9 instillations). QoL was measured using the EORTC QLQ-C30 v.03 questionnaires prior to the first and the last instillation of each BCG cycle. Group differences were calculated using linear regression corrected for T0 (baseline). Differences in QoL over time were tested for significance using a linear mixed model. Additionally, Chi-square tests were used to compare the number of side-effects between the two arms.

Results: We failed to detect any differences in the means of each QoL-scale between the two treatment arms at each time point ($p<0.05$). The linear mixed model also did not show any significant changes over time in all QoL domains for both arms ($p<0.05$). We did see significant differences in the incidence of general malaise ($p=0.03$) at T1, frequency ($p=0.002$), urgency ($p=0.02$), and dysuria ($p=0.001$) at T7 in favor of the reduced frequency arm.

Conclusions: Our study was unsuccessful in showing a better QoL in patients undergoing a reduced treatment regimen. This unexpected result may be explained by QoL loss because of more recurrences and QoL win because of less toxicity canceling each other out. Or by non-responsiveness of the EORTC QLQ-C30 questionnaire for small QoL changes.

The accuracy of cystoscopy in predicting detrusor invasion in newly diagnosed bladder cancer patients

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Introduction: The prognosis of muscle-invasive bladder cancer (MIBC) has not improved during the last three decades. Transurethral resection of the bladder tumor (TURBT) is the standard procedure for local tumor staging. Studies have shown TURBT to have a substantial number of limitations, including the spread of circulating tumor cells (CTCs). This urges the development of an alternative local staging strategy. We aimed at expanding the available evidence on the accuracy of cystoscopy to predict muscle invasion in newly diagnosed bladder cancer (BC) patients.

Methods: We included 304 newly diagnosed BC patients from one of 6 collaborating Dutch hospitals between July 2020 and February 2022. Predictions of detrusor muscle invasion using a 5-point Likert scale alongside the histopathology data were recorded. The sensitivity, specificity, predictive values, and 95% confidence intervals were determined using a standard contingency table.

Results: 217 (72.4%) of the confirmed carcinomas received a histopathological diagnosis of non-muscle-invasive bladder cancer (NMIBC), 65 (21.6%) were classified as (muscle-invasive bladder cancer (MIBC), and 2 (0.7%) were unknown. Cystoscopy could predict detrusor invasion with a sensitivity of 69.2% (95%CI: 56.6-80.1), and a specificity of 90.2% (95%CI: 85.6-93.7). This corresponded to a positive predictive value (PPV) of 66.2% and a negative predictive value (NPV) of 91.3%.

Conclusions: Our study shows a moderate accuracy of cystoscopy to predict detrusor invasion. This result does not support cystoscopy to replace TURBT for local staging.

Oncological outcomes of patients with node positive disease following neoadjuvant chemotherapy and radical cystectomy for muscle-invasive bladder cancer: A multicenter observational study of the EAU Young Academic Urologists (YAU) urothelial carcinoma working group

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Introduction: Until recently, there was no recommended adjuvant therapy for patients with lymph nodes metastasis (ypN+) following neoadjuvant chemotherapy (NAC) and radical cystectomy (RC) for muscle-invasive bladder cancer (MIBC). To describe the oncological outcomes of ypN+ patients following NAC and RC for MIBC.

Methods: This collaborative retrospective study included 195 patients from 7 centers who underwent NAC followed by RC for MIBC between 2000 and 2019. Patients' demographics, clinical and pathological features were collected. Survival analyses were carried out with Kaplan-Meier estimates and a cox model was generated.

Results: The median age was 65 years (59-71). A total of 120 patients (62%) were pN1, 51 pN2 (26%) and 24 pN3 (12%). Adjuvant radiation therapy was given in 18 (9%), adjuvant chemotherapy in 40 (21%) and the remaining 137 (70%) patients were observed. The median follow-up time was 51 months (95%CI 44-62). Median times for recurrence-free survival, cancer-specific survival and overall survival (OS) were 18 months (95%CI 16-21), 47 months (95%CI 31-70) and 28 months (95%CI 22-34) respectively. On multivariate analysis, female gender (HR=1.5, 95%CI 1.002-2.21, p=0.049) and positive margins at the time of RC (HR=1.6, 95%CI 1.06-2.38, p=0.026) were the only independent predictor of OS. The type of adjuvant therapy did not impact OS (adjuvant chemotherapy, p=0.44; adjuvant radiotherapy p=0.40).

Conclusions: MIBC patients with residual lymph node disease following NAC and RC have poor survival outcomes. We did not identify any benefit from adjuvant chemotherapy or RT. Females and patients with positive margin status at RC carry a poorer prognosis. These results may be beneficial for clinical trial design.

Chemotherapy and sequential immunotherapy for locally advanced urothelial cancer: the CHASIT study

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Introduction: Patients with locally advanced irresectable or clinically node-positive urothelial cancer (UC) have a poor outcome. Patients who experience adequate radiological response to induction chemotherapy (IC) and undergo resection of the primary tumor and affected lymph nodes thereafter, still have a chance of cure. Long-term survival strongly depends on the complete pathological response (pCR) rate, which is reported to be 15% following IC. The 5-year overall survival rates for patients achieving pCR is 70-80% versus only 20% for patients with residual disease (>ypT2N0) or nodal metastasis (>ypN0). This clearly demonstrates the unmet need to improve clinical outcome of these patients. The CHASIT study aims to assess the efficacy and safety of sequential chemo-immunotherapy in patients with locally advanced irresectable or clinically node-positive UC of the bladder, upper urinary tract or urethra. In addition, biomaterials (blood, urine and tissue) are collected to investigate biological mechanisms of response and resistance to immunotherapy. Keywords: muscle invasive urothelial cancer, immunotherapy, chemotherapy, pathological response

Methods: This multicenter, prospective phase II clinical trial includes patients with cT4NxM0 or cTxN1-N3M0 UC. Patients treated with 3-4 cycles of platinum-based chemotherapy and who do not experience disease progression on (PET)-CT-scan are eligible for inclusion. They receive 3 cycles of anti-PD-1 immunotherapy with avelumab (800mg/2weeks) followed by radical surgery. Follow-up consists of a CT-scan and blood and urine collection every 3 months until 2 years after surgery. We aim to demonstrate a pCR rate of 30% or higher. 64 patients will be screened to obtain a power of 80%. The primary endpoint is the pCR rate in the resected specimen. Secondary endpoints include toxicity, postoperative surgical complications, progression-free survival, cancer-specific survival and overall survival.

Results: n.a.

Conclusions: If the CHASIT study shows that sequential chemo-immunotherapy leads to a pCR rate of 30% or higher, a randomized controlled trial is foreseen to compare this new treatment regimen to standard care.

The Impact of Blue Light Cystoscopy Use Among Non-Muscle Invasive Bladder Cancer Patients in an Equal Access Setting: Implications to Recurrence, Time Interval to Recurrence, and Comparable Outcomes Stratified by Race

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Introduction: Use of blue light cystoscopy (BLC) and impact of bladder cancer outcomes among patients in an equal setting is largely unknown. To describe bladder cancer outcomes and the impact of blue light cystoscopy among non-muscle invasive bladder cancer (NMIBC) patients in an equal access setting.

Methods: A total of 378 NMIBC patients within the Veterans Affairs that underwent blue light cystoscopy (BLC) from January 1, 2018 to December 31, 2020 were assessed. We used the Kaplan-Meier method to estimate event-free survival and Cox regression to determine the association between race and recurrence, progression, and overall survival outcomes.

Results: The median follow-up time from bladder cancer diagnosis was 40.7 months (3.4 years). A total of 378 patients who had complete data were included in the analysis, of which 43 (11%) and 300 (79%) were Black and White, respectively. A total of 194 (51%) and 52 (14%) patients were T1 HG or T1 with or without CIS versus CIS with or without TaHG or T1, respectively. There were 127 (34%) patients with TaLG only. A total of 239 (63%) patients underwent BCG therapy. There were 91 (42%) patients who recurred prior to BLC but did not recur after BLC while 56 (15%) patients recurred prior to BLC and recurred after BLC. Median time to first recurrence following BLC vs. WLC was significantly longer overall and across all time points (40 (33-NE) vs. 26 (17-39) months, $p<0.001$), respectively. There was no significant difference in recurrence (Hazard Ratio (HR) 0.83; 95% Confidence Interval (CI) 0.48-1.43), progression (HR 1.46; 95% CI 0.45-4.74), and overall survival (HR 0.69; 95% CI 0.29-1.65) according to Black vs. White race.

Conclusions: In this study from an equal access setting in the VA, we observed decreased recurrence and prolonged time interval to recurrence among BLC patients and no difference in any bladder cancer outcomes according to race.

Concurrent chemoradiation for muscle-invasive bladder cancer using 5-fluorouracil versus capecitabine

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Introduction: Capecitabine, an oral 5-fluourouracil (5-FU) prodrug, could serve as a suitable alternative for 5-FU as a radiosensitizer in concurrent chemoradiotherapy (CCRT). Avoiding the necessity of indwelling central venous infusional devices, capecitabine is associated with increased convenience for both patients and healthcare professionals. Since large comparative studies are lacking, we aimed to compare toxicity, health-related quality of life (HRQoL) and survival after CCRT with 5-FU versus capecitabine.

Methods: Patients with non-metastatic urothelial MIBC (cT2-T4a, N0-2, M0), diagnosed between November 2017 and November 2019 and treated with CCRT with mitomycin C and either capecitabine or 5-FU were identified from the Netherlands Cancer Registry. Patient, tumor and treatment characteristics, and toxicity were compared between groups using ANOVA and Chi-square or Fisher-exact tests. HRQoL per treatment group was evaluated over time using the EORTC-QLQ-C30 questionnaire. Overall survival (OS) and disease-free survival (DFS) were evaluated using Kaplan Meier analyses with inverse probability treatment weights to adjust for baseline differences.

Results: Of the 266 patients included, 120 (45%) were treated with 5-FU and 146 (55%) with capecitabine. Compared to 5-FU, more patients in the capecitabine group completed a curative CCRT protocol according to treatment plan (60 vs 75%, p=0.01). Adverse events rates were equal in both groups (22 versus 18%, p=0.44). Two-year overall survival was significantly in favor of capecitabine (76% vs 62%, p=0.01). Two-year DFS was not significantly different (63% vs 51%, p=0.16). Reported HRQoL was similar for both treatment groups and showed a temporary decrease in several functioning or symptom scores at 6 or 12 months after diagnosis, but restored again to pre-treatment levels two years after diagnosis.

Conclusions: Concurrent chemoradiotherapy treatment with capecitabine and MMC is associated with a similar toxicity profile and health-related quality of life compared to 5-FU plus MMC. Also survival appears to be similar or even slightly better. As capecitabine is more patient-friendly compared to 5-FU, our data are in favor of replacement of 5-FU by capecitabine in CCRT of MIBC.

BLCA-RegMap portal: a co-regulatory influence network view of bladder cancer heterogeneity and plasticity

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Introduction: Bladder cancer studies performed in a variety of laboratories and by a number of large-scale projects have given an unparalleled amount of information on tumors and in vitro models. Consolidating these data into an easily accessible and comprehensive system-level format is crucial to accelerate systems oncology model development.

Methods: We combined network biology with machine learning and visualization techniques to execute a cycle of systems oncology model development: inference of the co-regulatory networks (from transformed cells in vitro), interrogation of the tumors in vivo using the inferred networks, and intervention with the network (feeding back to the in vitro tumor models). First, analysis of the in vitro gene expression profiles of 36 bladder cancer cell lines of the CCLE with CoRegNet yields to the reconstruction of the first co-regulatory influence network (BLCA-CoRegNet) that contains 359 TFs/co-TFs linked by 508 significant co-operativity interactions and regulating 6,374 targets genes. Second, BLCA-CoRegNet based influence map (BLCA-RegMap), is built using a meta-cohorts of > 2500 tumours and >100 cell lines transcriptome profiles.

Results: We introduce BLCA-RegMap a powerful web-based tool to help researchers to rapidly access a co-regulatory influence network view of bladder cancer heterogeneity and plasticity. BLCA-RegMap allow user to: explore the similarities and differences between cancer subtypes and identify their possible core regulators; identify rare subtypes; align tumor and cell line transcriptional profiles; and define new targets related to the different states and plasticity of the tumours (undifferentiated vs differentiated, therapy sensitive vs resistant cells, etc.). BLCA-RegMap has a very intuitive interface, and no bioinformatics skills are required. For all the networks and plots that are generated, the user can run different annotation (classification, genomic alteration and clinical), add new transcriptome data, and the raw data to reproduce the plots can be downloaded for future analysis or publications.

Conclusions: The identification of regulatory networks and the study of their plasticity should allow to identify efficient therapeutic strategies and will pave the way for precision oncology.

Dataset preparation to predict response to BCG treatment for high-risk non-muscle-invasive bladder cancer from histopathological images

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Introduction: Non-muscle-invasive bladder cancer (NMIBC) comprises 75% of newly diagnosed BC patients. The risk of progression to MIBC or metastasis is based on clinicopathological evaluations. Despite treatment with BCG, 50% of high-risk NMIBC (HR-NMIBC) tumors are not sensitive to treatment. Current risk stratification systems are partially limited due to inter- and intra-observer variability in pathological evaluations that can be improved using accurate and reproducible digital pathology (DP) tools. However, DP is currently limited by a lack of clinically representative datasets with adequate follow-up. Therefore, we aimed to provide an extensive clinically representative whole slide image (WSI) dataset from HR-NMIBC BC patients with long-term clinical follow-up.

Methods: In this multicentre retrospective study, HR-NMIBC patients who received ≥5/6 BCG induction instillations between 2000-2018 were included, and primary H&E slides were scanned. During quality control (QC), WSIs with out-of-focus regions were re-scanned. A pathologist classified all slides for grade, stage, and CIS, which, together with follow-up information, were considered weak labels for WSIs. Strong labels were created by delineating predictive areas for progression on a WSI – e.g., grade, stage, and CIS – and were confirmed by a pathologist. A consensus was made to annotate at least 20 strong labels per WSI.

Results: Clinical data and H&E slides were collected from 750 HR-NMIBC patients, from whom 792 WSIs were acquired. A total of 696 WSIs and 96 WSIs were provided with weak and strong labels. Initially, fully annotating a WSI took 15 hours on average. Annotating 20 strong labels for each WSI reduced the average time to 90 minutes. The average time from slide for scanning, pseudonymizing, annotating, confirming labels and QC was roughly 6 hours per WSI.

Conclusions: Here, we provide an extensive dataset that could be applied to develop clinically translatable DP tools predicting clinical outcome from primary BC WSIs. When looking into DP aspects, the time to prepare a WSI dataset should be considered. As fully annotating is not feasible, we created rough (90 mins/WSI) and detailed annotation (15 hrs/WSI) datasets and will compare them.

Exploring relationships between FGFR3 and EGFR growth factor signalling networks in normal human urothelium to better understand luminal and basal bladder cancer

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Introduction: Activating mutations of the fibroblast growth factor receptor 3 gene (FGFR3) are common in urothelial bladder cancer, where they are typically associated with less aggressive disease and a luminal phenotype. By contrast, amplifications of the epidermal growth factor receptor gene (EGFR) are associated with basal tumours. Urothelial EGFR signalling is usually autocrine whilst FGFR signalling is typically paracrine. Using an in vitro human urothelial cell culture platform, our aim was to identify the context(s) in which EGFR and FGFR3 receptors are available to drive autocrine- or paracrine-regulated proliferation.

Methods: Finite Normal Human Urothelial (NHU) cell lines were established in serum-free culture from surgical tissue.

Results: Activation of EGFR in absence of exogenous ligand was observed in agreement with previous findings that NHU cells express amphiregulin, which activates EGFR in an autocrine manner to promote proliferation (Varley et al., 2005). NHU cells did not express FGFR3 transcript or protein. Following inhibition of EGFR using PD153035 for 48-72h, FGFR3 protein was detected. RNA-sequencing showed that NHU cells did not express transcripts of ligands capable of activating FGFR3. Stimulation of FGFR3-expressing NHU cells with exogenous FGF9 resulted in phosphorylation of the FGFR3 downstream targets FRS2 and ERK.

Conclusions: The constitutive EGFR-FGFR3 signalling balance in the urothelium is dominated by autocrine EGFR signalling, however perturbing this by inhibition of EGFR resulted in FGFR3 expression. This FGFR3 is functional and can be activated by exogenous ligands, implying a paracrine role. In addition, analysis of the Cancer Genome Atlas bladder cancer cohort showed that amplification of EGFR was mutually exclusive with FGFR3 activation, suggesting different routes of alteration acquisition. Future work will employ phospho-proteomics to investigate the relationship between FGFR3 and EGFR in the urothelium, and how their downstream signalling cascades influence cells in a normal and cancer context.

Immune Cellular/Molecular Dynamics and Subtype Multiplicity of BBN-induced Bladder Tumor Development

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Introduction: Murine bladder cancers induced by the carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) closely mimic human bladder cancers, microscopically and molecularly. Accordingly, BBN-induced tumor development may provide actionable insights into human bladder cancers. Using single cell RNA sequencing and immunohistochemistry for discovery and validation, we analyzed wild type murine bladders at different timepoints of BBN exposure to discover key changes during carcinogenesis.

Methods: Female C57BL6 mice were exposed to control 5% dextrose or BBN 0.05% in the ambient drinking water for up to 20 weeks. Tumors were periodically assessed by in vivo micro-ultrasound. At 5, 10, 17, and 23 weeks after the start of BBN exposure, bladders from control and BBN-exposed subjects were harvested and fixed for 24 hours in 1% formalin for subsequent embedding in paraffin to allow histological examination or immediately processed into a single cell suspension for scRNA-Seq according to a 5' 10x Genomics workflow. Four micron sections cut from FFPE blocks were stained with immunohistochemistry (IHC) antibodies and positivity quantified with QuPath software. scRNA-Seq statistics were performed on R and Monocle with data visualization on UMAPs. IHC statistics were analyzed in GraphPad.

Results: Single cell genomic analyses of pooled BBN-exposed bladders showed expression of several intrinsic subtype gene signatures including basal, luminal, and squamous, suggesting subtype multiplicity. Molecules associated with immune exhaustion, AR and NR4A1, increased in T cells over time, with progenitor exhausted T cells particularly enriched for AR signature genes. IHC demonstrated the presence of tertiary lymphoid structures containing CD20+ B cells and CD8+ T cells after BBN exposure. Stromal HIF1α and epithelial TGFβ increased with longer durations of BBN exposure.

Conclusions: The BBN-induced murine bladder cancer model may be generalizable to a broader spectrum of human bladder cancers than previously thought, based on intrinsic subtype gene signatures. T cell exhaustion may be an immune factor that is permissive for bladder carcinogenesis. HIF1α and TGFβ oncoproteins are potential preventive or therapeutic targets in this model.

Elucidating the tumor suppressor role of STAG2 in bladder cancer

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Introduction: STAG2, a cohesin component involved in chromosome organization and transcriptional regulation, was identified by our lab, and others, as a bladder cancer (BC) tumor suppressor. Most STAG2 mutations are inactivating and associate with luminal tumors of low stage and grade. Accumulating evidence indicates that STAG2 loss is an early genetic event that participates in bladder carcinogenesis through mechanisms distinct from chromatin segregation.

Methods: To assess how STAG2 loss contributes to BC development, we generated a conditional knockout (KO) mouse model and established mouse bladder organoids and immortalized cultures of primary murine normal urothelial cells (NU1), both of which recapitulate urothelial differentiation. These tools allowed to conduct valuable genomic studies.

Results: Stag2 depletion in proliferating NU1 cells has minor transcriptomic effects but it has higher impact in differentiated cells, consistent with an enrichment in STAG2 localization at promoters and enhancers using ChIP-Seq. Although cell cycle genes are significantly upregulated in differentiated NU1 cells upon STAG2 loss, Stag2-knockdown cells do not show increased proliferation. Stag2 inactivation in the urothelium (Upk3a-CreERT) results in the upregulation of cell cycle genes and KI67 expression, both in Stag2-null urothelial cells and Stag2+ cells from the stroma, which hints at non-cell autonomous effects of STAG2. Preliminary data suggest that changes in transcription factor positioning, rather than altered chromatin accessibility, account for cell cycle gene upregulation. While STAG2 loss in the murine urothelium does not have major histological effects in homeostatic conditions, concomitant STAG2 deletion and cyclophosphamide-induced urothelial damage lead to hyperplasia and Stag2 KO urothelial cells display higher organoid-forming capacity.

Conclusions: In summary, Stag2 inactivation in the urothelium is not enough to trigger neoplastic transformation but results in cell cycle gene upregulation and primes cells for proliferation upon damage.

Control of RB/E2F1 expression level enhances the oncolytic potency of the oncolytic virus XVir-N-31

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Introduction: Oncolytic viruses and CDK4/6 inhibitors (CDK4/6i) are promising therapeutic agents for the treatment of various cancers. As monotherapy, replication potency of oncolytic viruses in solid tumors is limited and novel strategies to overcome these limitations are necessary to improve therapy response. As single agents, CDK4/6 inhibitors that are approved for the treatment of breast cancer in combination with endocrine therapy cause G1 cell cycle arrest, whereas adenoviruses induce progression into S-phase in infected cells as an integral part of the adenoviral life cycle. Both, CDK4/6 inhibitors and adenovirus replication target the Retinoblastoma protein even though for different purposes.

Methods: Different established and newly generated adenoviral vectors and reporter constructs were used in this study to examine the effect of viral replication, gene and protein expression, cell killing, particle formation in the combination with CDK4/6 inhibitors Palbociclib, Ribociclib and Abemaciclib. As tumor entities, bladder cancer cell lines and a murine sarcoma model were analyzed. Cellular protein expression was manipulated by using siRNA, shRNA and transfection of cDNA encoding vectors. Electron microscopy was applied for detecting viral particle numbers in cells.

Results: The combination of CDK4/6 inhibitors and the oncolytic adenovirus XVir-N-31 potentiate the anti-tumor effect in bladder cancer and murine Ewing sarcoma xenograft models. This effect correlates with an increase in virus-producing cancer cells, enhances viral genome replication, particle formation and consequently cancer cell killing. The molecular mechanism that regulates this response is fundamentally based on the reduction of Retinoblastoma protein and E2F1 expression levels by CDK4/6 inhibitors.

Conclusions: The combination of oncolytic adenovirus and CDK4/6 inhibitors is a promising novel strategy to significantly improve therapy response in cancer.

ATM/ATR as therapeutic targets to overcome resistance in platinum-adapted bladder cancer cells

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Introduction: Platinum-based chemotherapy remains the standard of care for advanced urothelial carcinoma of the bladder (UCB). Several clinical studies have shown that mutations in DNA damage repair genes (DDR) confer cisplatin sensitivity, thus improving survival. DDR checkpoints, including ATM-Chk2 and ATR-Chk1, are crucial mediators of therapy resistance in solid tumors. ATM/ ATR are therefore promising targets to overcome platinum resistance in UCB.

Methods: Cisplatin- and gemcitabine-resistant UBC cell lines (T24, RT4, RT112, TCCSUP, 5637, HT1376) were used after long-term adaptation to chemotherapy. Inhibition of ATM and ATR was achieved by specific inhibitors and shRNA knockdown. CRISP/Cas was used for ATM knockout in T24 cells. Expression studies were performed by WB and RT-qPCR. Cell viability was assessed by MTT assay and soft agar assays.

Results: Pharmacologic inhibition of both ATM and ATR resulted in a dose-dependent reduction in proliferation of chemoresistant cell lines. Cisplatin-resistant cells were particularly vulnerable to ATR / ATM inhibition and the IC50 was significantly lower in cisplatin-resistant cells than in gemcitabine-resistant cells and chemo-naive control cells indicating a cisplatin-specific effect. ATM knockout and ATR knockdown, respectively, resulted in significant reduction in cell viability of cisplatin-resistant T24 cells in vitro.

Conclusions: ATM/ ATR inhibition significantly enhanced response to cisplatin in platinum-resistant UCB cells in vitro. The results were confirmed by specific ATM knockout and ATR knockdown assays, respectively. Temporary DDR inhibition may therefore be a promising therapeutic strategy for chemosensitizing in patients with UCB receiving platinum-based chemotherapy.

Ex vivo modelling of carcinogenesis using the porcine urinary bladder (PUB) as a versatile tool for stem cell differentiation

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Introduction: Pluripotent stem cells (PSC) provide a valuable tool to study not only embryonic development but also carcinogenesis. However, PSC-derived cells often lack final maturity marker expression and need to be transplanted into living hosts to overcome this hindrance. Unfortunately, such approaches are labor- and cost-intensive. Here, we propagate the porcine urinary bladder (PUB) as an ex vivo organ culture model to address these limitations.

Methods: Marker gene expression was analyzed by qRT-PCR. PUBs were enzymatically and mechanically de-epithelialized before cell seeding. Immunohistochemistry and immunofluorescence were performed to confirm marker expression of engrafted cells.

Results: Directed differentiation of PSC toward the urothelial lineage and subsequent organoid culture results in urothelial marker expression (KRT20, UPK3). Engraftment of pre-differentiated uro-epithelial-like cells on-PUB resulted in a re-epithelialization and increased marker expression of urothelial cells (CK-20, FOXA1, UPK1B). Finally, we tested the suitability of the PUB to investigate carcinogenesis. To do so, we employed our recently published protocol to drive differentiation of PSC-derived pancreatic duct-like organoids (PDLO). Intriguingly, featuring oncogenic KRAS mutations, PDLOs on-PUB demonstrate signs of early dysplasia during pancreatic carcinogenesis. In addition to papillary tumor growth, dysplastic marker expression (CA19-9) was observed. Pancreatic cancer patient-derived organoids in combination with stromal components and immune cells propagated quickly to viable tumors on-PUB.

Conclusions: We here demonstrate the applicability of the PUB to function as a versatile, easy-to-access and cheap tool and organ culture model which supports stem cell differentiation and maturation and enables modelling of tumor onset and progression during early and late timepoints in carcinogenesis. In addition, we provide proof-of-concept data to study carcinogenesis by using PSC-derived systems with inducible oncogenes. Thus, we extend the spectrum of the current model systems for the investigation of carcinogenesis.

Characterization of T cells during BCG therapy for NMIBC

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Introduction: Despite having been used for decades in routine clinical practice, the mechanism underlying the antitumor effect of BCG in the context of NMIBC is not yet fully understood. We investigate the role of T cells in the context of BCG therapy. We track the frequency of T cells during treatment and try to determine their specificity. In particular, we try to determine if BCG therapy induces or reactivates tumor-specific T cells.

Methods: We collected tumor, blood and urine samples from 4 bladder cancer patients, before, during and after induction BCG treatment. We extracted the T cells from these samples, and performed single-cell RNA sequencing. Then, we analyzed the frequency and the phenotype of extracted T cell clonotypes in each sample and we selected clonotypes of interest. Next, we expressed the TCRs (T cell receptors) of the selected clonotypes in a reporter cell line and screened them for recognition of mutant peptides (neoepitopes) predicted from RNA-seq and WES (whole exome sequencing) data from the tumor of the patients.

Results: Of the 4 patients, 3 had pT1 HG and 1 had CIS. All have been followed over 1 year and none had a recurrence. For the patient with CIS, the induction treatment was very effective. In this patient, we found multiple new CD8+ T cell clonotypes present at a high frequency in the bladder after treatment. In all 4 patients, none of the selected clonotypes that were present after but not before the treatment recognized mutant peptides. In one patient with pT1 HG, we detected neoepitope-specific T cells, which were already present in the tumor before BCG. Thus, this patient had mounted a tumor-specific T cell response prior to BCG. To detect antigens other than neoepitopes, we set up screenings of tumor cDNA libraries. We are currently performing these screenings to further determine the specificity of the selected T cells.

Conclusions: In this study, we found 1 patient with pT1 disease with a spontaneous anti-neoepitope T cell response. In 1 patient with CIS who responded very well to the BCG induction treatment, we found multiple CD8+ T cell clonotypes present at a high frequency in the bladder. We are currently determining the specificity of these T cells by screening tumor cDNA libraries.

Survey of B and Plasma Cells in Bladder Tumor Microenvironments

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Introduction: Little is known about tumor infiltrating B cells in settings of bladder cancer (BCa), especially their roles in different tumor microenvironments. In this study, we analyzed bulk RNA sequencing from the Cancer Genome Atlas (TCGA) and single cell (sc) RNA sequencing(seq) data from BCa tumors (n=18), Bca PBMC (n=16), and healthy donor PBMC (n=3) to profile B and plasma cells (PCs).

Methods: scRNAseq data were imported into R (v4.03) using Seurat (v4.0.1). Genes expressed in fewer than 3 cells, cells with <200 or >2500 unique genes, and cells containing >15% mitochondrial genes were discarded. Known markers (MS4A1, IGHD, IGHM, IGHG1/2/3/4, IGHA1/2, MZB1, XBP1) were used to identify B cell and PCs.

Results: Within 15,008 naïve and memory B cells and PCs, we observed a proportional decrease in memory B cells in BCa tumor and PBMC compared to healthy PBMCs. The high percentage of naïve B cells in BCa PBMC an arrested development and maturation at the memory B cell stage, while the higher proportion of PCs in BCa tumor suggest potential arrested development pathways between PCs and memory B cells from germinal center (GC) B cells. We also observed significantly increased expression of ribosomal genes in BCa PBMC memory B cells and PCs compared to healthy PBMC, and tumor memory B cells and PCs showed significantly higher expression of IGHG1/2/3/4 and IGHA1 compared to BCa PBMC. Finally, we observed strongly protective effects of higher mRNA transcripts encoding IgG1 and IgG3 (isotypes involved in induction of antibody-dependent cellular cytotoxicity (ADCC)) and NK cells (NCR1 and NG7) in the BCa TCGA cohort, whereby MIBC patients with her transcripts for NK cells and IgG1 and/or IgG3 had significantly improved overall survival.

Conclusions: In this study, we observed significant proportional differences in B cell and PCs across tissue types that could possibly be attributed to a stall in maturation from naïve to memory B cell in the BCa blood or competing maturation pathways between PCs and memory B cells from GC B cells in BCa tumor. We also observed significant increases in genes supporting upregulation of immunoglobulin production both in tumor and in BCa blood relative to BCa PBMCs and healthy PBMCs, respectively. We found in advanced MIBC, that signatures of ADCC are predictive of improved overall survival.

Immune Microenvironment Profile of Clinically Aggressive Bladder Cancer Variants

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Introduction: In addition to conventional urothelial carcinomas (UCs), many microscopically distinct bladder cancer variants have been described. The most frequent of these variants are sarcomatoid, small cell, micropapillary, and plasmacytoid, which are more aggressive than conventional UCs. We aim to elucidate the molecular composition of immune microenvironment of these variants.

Methods: Our bladder cancer cohorts included conventional UC (n=84), micropapillary (n=43), sarcomatoid (n=28), small cell (n=22), and plasmacytoid (n=12) variants. A cohort of 408 bladder cancers in The Cancer Genome Atlas (TCGA) was used as a reference. Genomic expressions were analyzed by the Illumina's DASL platform. Immune infiltrates of B, T, CD8, MacTH1, and dendritic cell clusters were evaluated by DAVID. Additional analysis was performed by CIBERSORT to assess 22 immune cell types. These analyses were complemented by the expression profiles of 78 regulatory genes of the immune system.

Results: Genomic expression analysis of conventional UCs in the MD Anderson and TCGA cohorts demonstrated that they were classified into luminal, basal, and double negative subtypes. The basal and double negative subtypes in both cohorts were enriched for immune infiltrates (64.2 and 76.9% in the MD Anderson cohort; 54.1 and 76.4% in the TCGA cohort). While micropapillary and plasmacytoid variants expressed the signature luminal genes, sarcomatoid and small cell variants expressed distinct genes characteristic of the basal subtype. Immune infiltrate was enriched in sarcomatoid variant and 81.2% were immune hot. In contrast, small cell carcinomas were depleted of immune infiltrate and 86.4% were immune cold. In addition, 39.5% of micropapillary and 75% of plasmacytoid variants were immune hot. The UC variants also showed overexpression of unique immune regulatory proteins, including ADORA2A (a T cell inhibitor) in small cell carcinoma variant, PDCD1LG2 (program cell death 1 ligand 2) in sarcomatoid carcinoma variant, VTCN1 (a T cell inhibitor) in micropapillary variant and SELP (a regulator of innate immunity) in plasmacytoid variant.

Conclusions: Bladder cancer variants demonstrate distinct immune microenvironment profiles, which may represent targets for immune therapy.

Intravesical instillation of Ty21a/Vivotif: a new treatment for NMIBC patients?

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Introduction: Standard of care immunotherapy of non-muscle-invasive bladder-cancer (NMIBC) with intravesical Bacillus-Calmettte-Guérin (BCG) is associated with adverse events (AE), disease recurrence/progression and supply shortages. Preclinical data showed that intravesical instillation of Ty21a/Vivotif, the oral vaccine against typhoid fever, may be an effective and safer alternative to BCG. The aim of our study was to assess the safety of intravesical Ty21a in NMIBC patients with low/intermediate risk, not requiring BCG immunotherapy.

Methods: In this open label phase I dose-escalating study, 15 NMIBC patients were prospectively recruited between May 2018 and May 2021 at our institution. In phase Ia, a traditional 3+3 dose-escalation trial was performed, with weekly instillations for a total of 4 weeks. In phase Ib, ten patients received the selected dose, six times, once a week. Urinary culture was performed and AEs were collected at each instillation.

Results: In phase Ia, 5 patients were enrolled: 3 patients received weekly the starting minimal dose of 1 x 108 CFU for four weeks with 2/3 mild AEs, 1 patient received a 5-fold higher dose (5 x 108 CFU) experiencing a strong inflammatory syndrome 3 days after the instillation with a spontaneous remission within 48 hours and a fifth patient received an intermediary dose (2.5 x 108 CFU) with a persistence of 1 x 106 CFU/ml of Ty21a bacteria in the urinary culture one week after the instillation, treated with antibiotics because of mild local symptoms. Based on these AEs, 1 x 108 CFU of Ty21a was considered as the maximal tolerated dose for phase Ib and was instilled in 10 patients once a week for 6 weeks. All patients completed their treatment. Most of them experienced minor systemic AEs (mainly general malaise), while half reported mild local bladder AEs (Ty21a-induced cystitis, hematuria and frequency). AEs only occurred during 1-2 instillations for 40 % of the patients. Ty21a bacteria were only recovered in 3/72 urinary samples one week after instillation. No severe systemic AEs and no fever > 39°C were observed.

Conclusions: Intravesical Ty21a might be better tolerated than BCG with less cumulative side-effects, no fever > 39°C and lower risks associated to bacterial persistence. Ty21a treatment thus deserve future clinical trials to explore its potential anti-tumor efficacy.

Bladder immune responses upon intravesical Ty21a instillations in non-muscle invasive bladder cancer patients

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Introduction: Although BCG therapy is the standard treatment to treat NMIBC, repeated BCG treatments are associated with significant side effects and treatment failure. Moreover, BCG manufacturing shortage often occurred. Overall, this overall underlies the necessity for alternative or complementary new treatments. We therefore investigated another bacterial vaccine, the highly attenuated Salmonella enterica serovar Typhi strain, Ty21a, which has showed pre-clinical evidences for its safe intravesical use, as well as for inducing T-cell- and dendritic cell (DC)-mediated tumor-regression in mice. In a recent a Phase I trial, we demonstrated the good safety profile of intravesical instillations of Ty21a in NMIBC patients and here, we assessed its immunogenicity.

Methods: 14 patients with low grade NMIBC were prospectively included to undergo 6 weekly instillations of Ty21a after TURBT. Urinary cytokine concentrations were analyzed by Luminex® and immune cell infiltration by flow cytometry along Ty21a intravesical instillations.

Results: Ty21a, as compared to BCG, increased fewer inflammatory cytokines, though it also induced a local Th1 environment. Although a significant increase in total urine infiltrating cells was observed between pre and postTy21a samples, the urinary cell infiltration was similar among pre or postTy21a samples, and perhaps even tends to decrease after the fourth instillation, suggesting that repeated Ty21a instillations do not promote the accumulation of urinary immune cells. Nevertheless, similarly to BCG instillations, most of locally recruited cells were neutrophils. Monocytes, T cells, DCs and NK cells were also significantly increased, but only during the first instillations, suggesting that, in contrast to the BCG therapy, a lower number of Ty21a instillations might be required to achieve a maximal immune cell infiltration. Interestingly, Ty21a, but not BCG, induced the infiltration of urinary DCs, including conventional and cross-presenting DCs, which were associated to therapeutic efficacy in the mouse model.

Conclusions: Although limited by the relatively small population included and the low number of urinary cells recovered after the fourth Ty21a treatment, our study showed that Ty21a immunotherapy of NMIBC patients is able to induce immune responses with possible anti-tumor potentials.

Characterization of the tumor-infiltrating immune repertoire in muscle invasive bladder cancer

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Introduction: It has been suggested that Muscle-Invasive Bladder Cancer (MIBC) subtypes follow different tumorigenesis pathways playing decisive roles at different stages of tumor development and resulting in a tumor microenvironment containing both innate and adaptive immune cells (T and B lymphocytes). We aimed to characterize the MIBC tumor immune-microenvironment by analyzing the tumor-infiltrating B and T repertoire according to the taxonomic molecular subtypes.

Methods: RNAseq data from 396 MIBC samples included in TCGA were considered. The subtype information was collected from the international consensus taxonomic classification describing six subtypes: Basal/Squamous-like (Ba/Sq), Luminal papillary (LumP), Luminal non-Specify (LumNS), Luminal unstable (LumU), Stroma-rich, and Neuroendocrine-like (NE-like). Using MiXCR, we mapped the RNA read sequences to their respective B-cell (BCR) and T-cell receptor (TCR) clonotypes. To evaluate the BCR and TCR differences among subtypes, we compared diversity measures using a Wilcoxon test and we performed a network analysis to characterize the clonal expansion. For the survival analysis stratified by subtypes, adjusted Cox regression models were performed.

Results: Overall, we found different patterns of tumor-infiltrating immune repertoire among the different MIBC subtypes. Stroma-rich and Ba/Sq tumors showed the highest BCR and TCR infiltration while LumP showed the lowest. In addition, we observed that the Ba/Sq and Stroma-rich tumors were more clonally expanded than the Luminal subtypes. Moreover, higher TCR richness and diversity was significantly associated with better survival in the Stroma-rich and Ba/Sq subtypes only.

Conclusions: This study provides evidence that MIBC subtypes present differences in the tumor immune-microenvironment, in particular the Ba/Sq and the Stroma-rich are related with a higher tumoral-infiltrating immune repertoire, which seems to be translated into better survival. Determining the causes of these differences will help to improve our understanding of the disease and the distinct rate of response to immunotherapy of MIBC.

T Cell-to-Stroma Enrichment (TSE) score: a transcriptomic marker that predicts response to immune checkpoint inhibitors in urothelial cancer

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Introduction: Immune checkpoint inhibitors (ICIs) improve overall survival in patients with metastatic urothelial cancer (mUC). However, only a minority benefits from treatment, stressing the need to select patients for treatment.

Methods: In this study, 70 patients with mUC who started treatment with pembrolizumab were included. Whole-genome DNA-sequencing, RNA-sequencing and PD-L1 combined positivity score (CPS) was available for 70, 41 and 40 patients, respectively. Patients were classified as either responder (n = 24; response or stable disease at 6 months) or non-responder (n = 46) according to RECIST v1.1. We examined the genomics and transcriptomics for the discovery of new predictors for response to ICIs in patients with mUC.

Results: Response rate was higher for patients with high vs. low tumor mutational burden (TMB; 47% vs. 19%) and high vs. low/no APOBEC mutagenesis (48% vs. 24%). Other genomic features and PD-L1 CPS did not correlate with response to pembrolizumab. The transcriptome of responders vs. non-responders revealed alterations in pathways related to immune cells and stroma activation. Furthermore, hierarchical clustering of gene expression signatures representing immune cells and stromal cells identified three groups of patients (n = 15, n = 14 and n = 12) that correlated with response to treatment. We noticed that the abundance of T cells relative to stromal cells represented by the gene signatures recapitulates the three cluster of patients. This transcriptomic metric that we named the T cell-to-Stroma Enrichment (TSE) score showed that patients with a positive and negative TSE score had a progression free survival rate at 6 months of 67% and 0%, respectively. The predictive value of the TSE score was validated in two independent cohorts of n = 348 patients with mUC treated with anti-PD-L1 (IMvigor210) and n = 84 patients with muscle-invasive bladder cancer who received neoadjuvant anti-PD-L1 (ABACUS).

Conclusions: The TSE score represents a robust and clinically applicable marker that may aid to select patients with metastatic and primary UC for treatment with ICIs.

Urothelial Bladder Cancer microbiota identified using tumour RNA-Seq data

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Introduction: Urothelial Bladder Cancer (UBC) is the 4th most common cancer among men in Europe. While the UBC tumor microbome can be used for patient stratification and may have prognostic value, still its full characterisation remains pending.

Methods: We studied the bacteria sequences in 1) TCGA BLCA cohort (433 samples from 412 primary MIBC tumours and 21 normal adjacent bladder tissue), and 2) UROMOL study, (475 samples of primary NMIBC tumours). We processed the transcriptomic data from both studies following the same procedure whenever possible. In TCGA we kept read pairs and have a considerable the amount of reads, while in UROMOL most reads were singletons and library depth was smaller. Human reads were discarded, and quality filters applied (remove adapters, low complexity and reads shorter than 75 bp). Remaining reads were classified using Kraken2 against Bacteria database. We calculated richness (number of different species/sample) and estimated the bacterial load dividing by the number of host mapped reads. We tested microbiome variation according to sex, stage, classification (1, 2a, 2b, 3) subtype in UROMOL, and Consensus class in TCGA, progression-free and cancer-specific survival.

Results: A total of 42 bacterial species were detected in the tumours of UROMOL study, being the more abundant E. coli, E. faecalis and S. enterica. In contrast, the more abundant bacteria in the TCGA dataset were L. lactis, N. gonorrhoeae and C. acnes. Regarding the NMIBC subtyping, Class 3 had a greater abundance of B. thuringiensis than Class 1 and Class 2b ($p<0.00047$ and 0.0014). Class 3 differed to Class 1 in the abundance of L. plantarum ($p<0.00027$). In the MIBC dataset, two species were different by sample type: R. pickettii was more abundant in normal bladder tissue ($p<0.0032$) and M. silvanus was more abundant in the primary tumor samples ($p<0.0003$). S. melonis and Bradyrhizobium sp. SK17 were different in Consensus classes.

Conclusions: This study sheds light into the urinary microbiota present in NMIBC and MIBC from two cohorts of different of geographical setting, different library preparation and RNA-Seq pipelines. We are in the process to replicate and validate these results in large international clinical studies.

Integrated analysis of the bacterial microbiome, differential host gene expression and immune cell profile in the tumor microenvironment of non-muscle invasive bladder cancer

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Introduction: Recently, it has been shown that communities of bacteria (referred to as the bacterial microbiome) exists in both normal and cancerous tissues and can impact treatment efficacy (e.g., metabolizing chemotherapeutics). We have compared the urinary microbiome, the tumor microbiome in archival tissue and correlated this to differential host immune gene expression and immune cell profiles within the tumor microenvironment (TME).

Methods: Bacterial signatures in urine (n=56) and FFPE tissues (n=66), (matching patients n=44) were determined using 16s rRNA sequencing (V3-V4). Alpha and Beta diversity analysis was performed. Nanostring IO360 panel from tumor RNA was correlated to the tumor microbiome profile. 9-colour multiplex immunohistochemistry investigated immune cell type infiltration (CD4, CD8, CD68, CD57, FOXP3, GRZB, PD-L1, PANCK, DAPI), within the tumor tissues (n=71).

Results: Diversity and composition of the bladder tumor microbiome decreased with disease progression, becoming more homogeneous from pTa to pT1 to pT2. The bacterial profiles between urine and FFPE cancer tissues revealed independent groups, showing urine is not an accurate proxy or biomarker for the bacterial composition within the tumor. Immune cell profiles showed strong differences between stromal and tumor regions, and cytotoxic T cell (CD8+) showed higher expression in LG disease, whereas HG disease had increased levels of macrophages (CD68+), natural killer cells (CD57+) and PD-L1 expression.

Conclusions: The tumor microbiome in NMIBC has differential bacterial components, distinct from urine, altering from low grade to high grade disease and associated with degree and type of immune infiltrate. Modulation of the microbiome may have important therapeutic potential.

Infiltrated luminal muscle-invasive bladder tumors have favourable outcomes with neoadjuvant chemotherapy

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Introduction: Muscle-invasive bladder cancer (MIBC) is treated with neoadjuvant chemotherapy (NAC) followed by radical cystectomy (RC). Several recent studies have identified unique molecular subtypes which have distinct biological and clinical characteristics, including differential response to NAC. However, reports on the specific biology and sensitivity to NAC of tumors classified as "luminal" on the luminal-basal axis have been conflicting. Here, we provide a detailed characterization of luminal tumors as designated by the Decipher genomic subtyping classifier (GSC).

Methods: Microarray data from TUR tissue of a prospectively collected commercial MIBC cohort (n=548) and an inverse probability weighted cohort (NACmeta; Lotan et al., 2021) were analyzed. Gene expression signatures and molecular subtypes were assigned as previously described (Necchi et al., 2020). The Kaplan-Meier method was used to estimate statistical significance of differences between survival curves for patients within the NACmeta cohort. Statistical analysis was performed using R version 4.1.3.; all significance testing used a two-sided t-test at a threshold of 0.05.

Results: The GSC infiltrated luminal (IL) subtype had the most overlap with the TCGA Luminal infiltrated subtype (66%) and with the Consensus luminal subtypes (LumNS 43%, LumP 13%, LumU 27%). The GSC luminal subtype overlapped with the Luminal papillary subtype of both the TCGA (67%) and Consensus models (56%). Comparing the GSC luminal and IL subtypes, we found the IL tumors demonstrated higher scores for stromal, EMT and immune signatures. Drug-response-signature scores (DRS) for cisplatin or gemcitabine were significantly higher in IL (both $p<0.001$). In the NACmeta cohort, patients with IL showed favourable 3-yr overall (OS) and cancer specific survival (CSS) with vs without NAC (OS, 74% vs. 55%, $p=0.01$; CSS, 82% vs. 61%, $p=0.007$).

Conclusions: GSC IL is defined by robust expression of luminal and stromal genes, with modest expression of immune-associated genes. The high DRS for cisplatin and gemcitabine in IL tumors was reflected in favourable outcomes of patients with IL tumors treated with vs without NAC.

Remapping TCGA bladder cohort shows different basal immune responses and tumour heterogeneity

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Introduction: Since the publication of the muscle invasive bladder cancer cohort of The Cancer Genome Atlas (TCGA, 2017), the genome feature annotation has been revised 17 times, increasing the number of protein-coding alternative transcripts and improving long non-coding RNA annotations. By remapping the RNA sequencing (RNAseq) reads we sought to assess mutational heterogeneity and further resolve molecular subtypes.

Methods: Raw RNAseq reads were pseudoaligned to the GRCh38 Gencode v37 reference transcriptome using kallisto. The reads were also aligned to the reference genome to validate expression values, and to compare RNA sequencing-derived mutation calls relative to matched whole-exome sequencing. We then reclustered gene expression data, interpreting the classification using in vitro studies from normal urothelium.

Results: Gene expression values were highly congruent between the existing public data and our remapping, resulting in few consensus label changes. Multiple markers specific to the neuroendocrine-like group had the poorest correlations. Pseudoalignment enabled transcript-level quantification for the first time, confirming canonical transcript usage, such as the predominance of PPARγ1. Intriguingly, 149 genes which appeared unexpressed in the public data had high expression transcripts upon remapping and were also "missed" in other TCGA cohorts. After reclustering the TCGA's Basal/Squamous (Ba/Sq) group can be further split into three, consistent with an in vitro study of IFNγ response in the urothelium. The basal subgroups are characterised by a high, medium, and low IFNγ response, highly correlated with tumour purity and exhibiting significantly different survival ($p=0.0033$). Luminal tumours were largely unchanged by reclustering. Over 90% of mutations in expressed genes were identified from RNAseq data, but intra tumour heterogeneity was also observed. Above 35% of whole exome-derived mutations were in unexpressed genes from RNAseq reads.

Conclusions: Remapping identified missing genes in the public data, and tumour heterogeneity. The robust split in Ba/Sq subgroups characterised by heterogeneity in IFNγ signalling suggests new opportunities for managing immune checkpoint blockade in basal tumours.

Characterization of molecular stroma-rich muscle-invasive bladder cancer

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Introduction: Transcriptomic based molecular subtypes of muscle-invasive bladder cancer (MIBC) enhanced the understanding of MIBC biology. However, the reproducibility and therapeutic options of the molecular stroma-rich subtype of muscle-invasive bladder cancer remain uncertain. We aimed to characterize the tumor-microenvironment (TME) of stroma-rich MIBC compared to other molecular subtypes.

Methods: We determined gene expression profiles of 88 MIBC patients undergoing radical cystectomy using the HTG transcriptome panel with 19398 mRNA targets to assign molecular consensus subtypes. Histological re-evaluation was performed. Multiplex immunohistochemistry and imaging of TME (PD-L1, PanCytokeratin, aSMA, Vimentin, CD45, Ki67) and T-Cell (CD4, CD3, PD1, CD163, CD8, FoxP3) panels were used to detect and quantify stroma- and immune-cell populations.

Results: 19 of 88 (22%) patients had a stroma-rich molecular subtype, of which 79% of patients showed not otherwise specified (NOS), 16% micropapillary, and 5% squamous histological subtypes. The estimate immune score did not differ significantly between stroma-rich and other subtypes. However, in the morphological analysis, molecular stroma-rich tumors showed increased immune-cell infiltration, but lower levels of PD-L1 positive and proliferating immune cells. Calculated distances of T-regs and macrophages to other cells were significantly higher compared to other molecular subtypes.

Conclusions: For a deeper understanding, the stroma-rich molecular subtype might not sufficiently be reflected by bulk-RNA sequencing. A higher morphological and molecular resolution is needed to unravel interactions between tumor and stroma cells, which is important to advance treatment decisions based on (molecular) biomarkers.