Tenth European Workshop and InNerMeD Information Network

Third Open Conference

Madrid, Spain,
Rafaelhoteles Atocha

March 16th - 19th, 2016

European Task Force on Brain and Neurodegenerative Lysosomal Storage Diseases

Fondazione BRAINS FOR BRAIN- Onlus Via Giustiniani 3 c/o Dipartimento di Pediatria Salus Pueri - 35128 Padova - Italy
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Lysosomal Storage Disorders (LSDs) are inherited metabolic disorders due to the deficit of lysosomal enzymes causing accumulation of mucopolysaccharides which is responsible for cell apoptosis with time. Since lysosomal enzymes are ubiquitous molecules, their deficiency has important effects in all organs, in particular the central nervous system (CNS), liver, spleen, heart and bones.
With the advent of recombinant DNA technology, the identification and cloning of all the known lysosomal enzymes has been recently achieved, and therefore, expression and purification of recombinant proteins is now possible and enzyme replacement therapy (ERT) is now available for a growing number of storage disorders.

However, although ERT has proven to be valuable to possibly change the clinical history of the disease it has been evident that the recombinant proteins do not have any effect on the CNS, as they are unable to cross the blood brain barrier.
Furthermore, the mechanisms and etiology of CNS pathology in LSDs are still poorly understood.

We still do not know whether storage and accumulation of mucopolysaccharide is really the “primum movens” of the metabolic disaster or whether other processes might be more important (inflammation, alteration of ion channel activity, lack of chaperone molecules etc.). The understanding of these basic aspects might be extremely valuable to unravel why most of the LSDs have an attenuated and a severe form without and with CNS involvement, respectively, depending on whether there is a total enzymatic deficiency or not.

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THE BRAINS FOR BRAIN TASK FORCE

The task force takes advantage from the expertise of the most distinguished european scientists, leaders in basic and applied neurotechnology and neurology grouped together to create a coordinate effort toward the comprehension of the pathophysiological processes of the neurological disorders, the implementation of knowledge on the blood brain barrier and the development of new molecular and or biochemical strategies to overcome the blood brain barrier and treat neurological disorders.
The B4B nickname of the group has been created to acknowledge the effort of the 4 initial industrial sponsors (ACTELION, BIOMARIN, GENZYME and SHIRE Human Genetic Therapies) without the support of which this brainstorming pannel could not have been created.

Brains For Brain (B4B) was formally founded in March 2007 as a research group formed by international specialists and leaders on clinical and basic research in the field of neuro-pediatrics and neuroscience.
The group has attracted interest from major biotech companies working on the development of new therapeutical strategies for lysosomal diseases, and furthermore has a strong interaction with international family associations, involved in taking care of the needs of lysosomal patients, and has stimulated collaborations toward coordinate actions to disseminate knowledge about the diseases.

B4B has also collaborated with International Scientific Associations, such as the European Study Group for Lysosomal Diseases (ESGLD) and the International Blood Brain Barriers Society (IBBS) and it is a member of the European Brain Council.

THE BRAINS FOR BRAIN FOUNDATION

The BRAINS FOR BRAIN FOUNDATION is a no-profit international organization addressed to disabled children who are affected (or healthy carriers) by rare neurological diseases.
The purposes of the FOUNDATION are:
- scientific research;
- dissemination of knowledge;
- social and socio-medical assistance;
- health assistance.

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In the field of Neurodegenerative Lysosomal Disorders the aims of the FOUNDATION are:

- to support medical and scientific research with regard to paediatric rare neurodegenerative diseases (with particular regard to Lysosomal Storage Disorders and genetic pathologies);
- to increase public awareness and interest on such diseases;
- to organize and promote national and international research activities;
- to coordinate and promote preclinical and clinical trials;
- to organize conferences and workshops on the above mentioned topics;
- to share cultural and scientific backgrounds with different stakeholders to implement knowledge on Neurodegenerative Disorders;
- to raise funds to support research;
- to fund fellowships or prizes;
- to campaign to increase public and stakeholders awareness to Neurodegenerative Disorders and for public fund raising.

THE EUROPEAN PARLIAMENT MEETINGS

Rare neurological diseases of childhood pose a serious medical health issue in Europe. Although individually uncommon, collectively there are thousands of rare diseases that affect a large number of people. The need to collaborate to focus on these disorders was highlighted during the meeting: RARE NEUROLOGICAL DISEASES OF CHILDHOOD: WE TREAT THE CHILD TO TREAT THE ADULT organized by the Brains for Brain Foundation at the European Parliament in Brussels on December 2nd 2010. The main aim of the meeting was to acknowledge the growing interest of the European Union Commission in both rare and neurological disorders.

To reinforce the necessity to work together and center attention on rare neurological disorders of infants and children, the B4B Foundation has more recently organized the roundtable: “PAVING THE WAY FOR A COMPETITIVE AND DYNAMIC EU KNOWLEDGE ECONOMY: THE WAY FORWARD IN RARE DISEASES" which was held again at the EU Parliament in Brussels, on November 26, 2013. The meeting rallied numerous relevant stakeholders to discuss initiatives aiming to create a model of intersectoral cooperation that could facilitate the set-up of a European PhD Programme in the area of rare neurological diseases of children. In line with the core principles established by “Towards a Maastricht for Research”, the Brains for Brain Foundation has in fact created a network of Universities and Scientific Societies to start a doctorate programme aimed at furthering the knowledge on neurometabolic diseases amongst young physicians and scientists in order to establish an European Network of specialized experts and maintain

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excellence in Europe. Such initiative intends to enhance an advance awareness and knowledge about rare diseases via cross-border collaboration and to enable better diagnosis and management of patients affected by these diseases.
Holding these meetings, B4B wished to demonstrate the unity of intent of family associations, biotechnology and pharmaceutical industries and the scientific community in stimulating interest in rare neurological diseases and advance care for affected children.
The B4B EP Roundtable in particular represents a major step toward the establishment of a successful EU cross border collaboration and cooperation to raise awareness about rare diseases of childhood and keep them on the health-care agenda. Although individually rare by definition, rare diseases collectively affect millions of people worldwide. Joint forces to tackling them are essential to ensure that affected children are given the priority they deserve and that their needs are met.

INHERITED NEUROMETABOLIC DISEASES INFORMATION NETWORK

The Inherited NeuroMetabolic Diseases INFORMATION NETWORK (InNerMeD-I-Network) has been funded by the Executive Agency for Health & Consumers (DG-SANCO) under the Second Programme of Community action in the field of Health, 2008-2013 (contract id 20121212) to be the first European Network on inherited neurometabolic diseases (iNMDs).
InNerMeD-I-network wants to create a network of information targeted on diagnosis and treatment of iNMDs based on the collection and exchange of proper information among scientific community, health professionals, patients, patient associations and all interested stakeholders. The project aims to increase current knowledge on iNMDs and speed up the timely and precise identification of patients, who may benefit of the available (experimental and marketed) treatments. The network will also favour biomedical research, straightening research capacities and fostering innovative therapeutic tools derived from the recent scientific advancements based on biomarkers use and personalised approaches.
The InNerMeD-I-Network, coordinated by the Brains for Brain Foundation, includes four associated partners (Gianni Benzi Pharmacological Research Foundation, Center for Metabolic Disorders at the University of Copenaghen, University of Zagreb School of Medicine, Hospital Sant Joan de Déu) plus fifteen collaborating partners, including clinical and research centres, patients and parents associations and scientific societies.

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AIMS OF THE WORKSHOP

The aims of the tenth Meeting of the Brains For Brain Foundation are:

- to discuss research achievements in the field of neurodegenerative disorders at clinical and basic science level in the field of neurodegenerative lysosomal storage disorders and Blood Brain Barrier;
- to discuss new recent advances on natural history and pathophysiology of LSDs particular attention to the important role of an early intervention in preventing the morbidity and mortality associated with each of the disorders;
- to discuss factors which control the entry into the brain of medicines and other therapeutic agents which may be helpful in treating central nervous disease;
- to discuss how B4B might collaborate with the European Union to stimulate interest in the research on LSDs and BBB. For this reasons representatives from EU Commission will be invited;
- to discuss collaborations with international family associations and corporations to increase knowledge about storage diseases and research projects;
- to discuss the role of the industries in driving innovation for new therapeutical approaches for true unmet needs.

This Workshop arises from the project Inherited NeuroMetabolic Diseases Information Network (InNerMeD-I-Network, agreement no. 2012 12 12) which has received funding from the European Union, Executive Agency for Health and Consumers, in the framework of the Second Health Programme.

Organization
Maurizio Scarpa (IT), **David Begley** (UK), Coordinators
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SCIENTIFIC PROGRAMME 2016

March 16th 2016

14.20-14.30
WELCOME AND OPENING

BASIC ASPECTS 1

ORGANISER AND CHAIRS
MAURIZIO SCARPA, DE
AND DAVID BEGLEY, UK

14.30-15.15
OPENING PLENARY LECTURE
MARINO ZERIAL
Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, DE
Mechanisms of endosome biogenesis, fusion and signalling
Discussion

15.15-15.40
ROMEO CECCHELLI
University of Artois, Lens, FR
Reducing attrition rate: implementation of human *in vitro* BBB models into the flow of standard assays used in drug discovery
Discussion

15.50-16.15
RITVA TIKKANEN
University of Giessen, Giessen, DE
Revisiting Aspartylglucosaminuria (AGU): Characterization of structural consequences of novel and old mutations reveals prospects for therapy for AGU
Discussion

16.25 Coffee

BASIC ASPECTS 2

CHAIR DISCUSSANTS
ANTONIO FEDERICO, IT
AND MARIA VANIER, FR

16.45-17.10
JACQUELINE IMRIE
NPUK, UK
The Natural History of NPC reveals prospects for therapy for AGU
Discussion

17.20-17.45
BEGOÑA CACHÓN-GONZÁLEZ
University of Cambridge, Cambridge, UK
Successful treatment of Krabbe disease - the holy grail among hereditary leukodystrophies
Discussion

17.55-18.20
MIA HOROWITZ
Tel Aviv University, Ramat Aviv, IL
The Contribution of Mutant GBA to the Development of Parkinson's Disease in Drosophila
Discussion

18.30-18.55
ARI ZIMRAN
Shaare Zedek Medical Center, Jerusalem, IL
Making the case for Ambroxol for type 3 Gaucher Disease: a 15-year long saga with a new twist for use in Parkinson
Discussion

Dinner

March 17th 2016

PATHOPHYSIOLOGY

CHAIR DISCUSSANTS
MIA HOROWITZ, IL
AND HALDAR KASTURI, USA

9.00-9.25
BARRY BOLAND
University College Cork, IE
C-terminal Fragments of Amyloid Precursor Protein (APP): Fingerprints of Subcellular Storage in Neurodegenerative Diseases
Discussion

9.35-10.00
JON COOPER
King's College London, London, UK
The Nature of Glial Dysfunction and its Impact Upon Neurons Varies Between Forms Of ncl
Discussion

10.10-10.35
HELEN PARKER
University of Manchester, Manchester, UK
Mucopolysaccharidosis IIIA Storage Substrate Drives an Innate Immune Neuro-inflammatory Response
Discussion

10.45 Coffee

CHAIR DISCUSSANTS
ROMEO CECCHELLI, FR
AND REINHARD GABATHULER, CA

11.15-11.40
INGOLF BLASIG
Leibniz-Institut für Molekulare Pharmakologie, Berlin-Buch, DE
Tight junctions in the blood-brain barrier under stroke and claudin mimetics
Discussion

11.50-12.15
MATHEW CAMPBELL
Trinity College Dublin, Dublin, IE
Tight junction protein expression and neurological disease
Discussion

12.25-12.50
JOHANNES BERGER
Medical University of Vienna, Vienna, AT
The current model of why hematopoietic stem cell transplantation is more potent in halting the inflammation in X-linked adrenoleukodystrophy than in halting the disease progression in metachromatic leukodystrophy
Discussion

13.00 Lunch

DELIVERY SYSTEMS/CROSSING THE BBB

CHAIR DISCUSSANTS
GERT FRICKER, DE
AND JÖRG KREUTER, DE

14.30-14.55
SYLVIA WAGNER
Fraunhofer Institute for Biomedical Engineering, Sulzbach, DE
Uptake mechanism of ApoE-modified nanoparticles
Discussion

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15.05-15.30
SVETLANA GELPERINA
Drugs Technology Ltd, Moscow, RU
Nanoparticle-bound Doxorubicin for chemotherapy of Glioblastoma: overcoming barriers
Discussion

15.40-16.05
PIETER GAILLARD
2-BBB Medicines BV, Leiden, NL
Safely enhancing brain drug delivery using the G-Technology in clinical programs targeting brain cancer and neuro-inflammation
Discussion

16.15 Coffee

CLINICAL APPLICATION

CHAIR DISCUSSANTS
ARI ZIMRAN, IL
AND JOHANNES BERGER, AT

16.45-17.10
MARC TARDIEU
Hôpitaux Universitaires Paris-Sud, Le Kremlin Bucêtre, FR
Intra-cerebral administration of AAV vector containing the human alpha-N-acetylglucosaminidase cDNA in children with Sanfilippo type B (MPSIIIB) syndrome: results at 12 months of a phase I/II trial
Discussion

17.20-17.45
STEFAN SVENSSON GELIUS
Swedish Orphan Biovitrum, Stockholm, SE
Intravenously administrated enzyme replacement therapy in the MPSIIIA male mouse - reversal of lysosomal storage and inflammation in the brain with glycan modified sulfamidase
Discussion

17.55-18.10
HILA ZIGDON
Weizmann Institute of Science, Rehovot, IL
Delineation of neuronal pathological events in a chemically-induced mouse model of Gaucher disease
Discussion

18.20-18.45
KASTURI HALDAR
Boler-Parseghian Center for Rare and Neglected Diseases, University of Notre Dame, Notre Dame, USA
Chronic administration of an HDAC inhibitor treats both neurological and systemic Niemann-Pick type C disease in a mouse model
Discussion

Dinner

March 18th 2016

CLINICAL APPLICATIONS 2

CHAIR DISCUSSANTS
MAURIZIO SCARPA, DE
AND TIMOTHY COX, UK

PLENARY SESSION TO MARK THE RETIREMENT OF DAVID BEGLEY

9.00-9.35
JÖRG KREUTER
Goethe University, Frankfurt, DE
David Begley and Nanoparticles
Discussion

9.45-10.20
ANDREAS REICHEL
Drug Discovery DMPK, Bayer Pharma AG, Berlin, DE
Pharmacokinetics of Blood-Brain Barrier Transport
Discussion

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Coffee

10.45-11.20
STEFANIE D KRÄMER
Institute of Pharmaceutical Sciences, ETH Zurich, CH
Physico-chemical Considerations for Small Molecule entry to the CNS
Discussion

11.30-12.05
MAURIZIO SCARPA
Brains for Brain Foundation, IT and Centre for Rare Diseases at the Helios Dr. Horst Schmidt Kliniken, Wiesbaden, DE
Surprise talk
Discussion

12.15-12.50
ANNE ILTZSCHE
Kings College, London, Blood-Brain Barrier Group, London, UK
Uptake and distribution of ApoE-targeted human serum albumin nanoparticles and their delivery to neurons in the mouse brain
Discussion

13.00 Lunch

BRAINS FOR BRAIN AND INNERMED EUROPEAN ACTIONS

CHAIR DISCUSSANTS
MAURIZIO SCARPA, DE
AND DAVID BEGLEY, UK

14.30 -14.50
ANTONIO FEDERICO
European Academy of Neurology
The role of the European Neurological Society in the promotion of research and care of Rare Neurologic Diseases
Discussion

15.00-15.20
SAMANTHA PARKER
European Committee of Experts on Rare Diseases (EUCERD)
Effective European Policy for Rare Diseases
Discussion

15.30-15.50
MATT JOHNSON
European Organisation for Rare Diseases (EURORDIS)
Toward the European Reference Networks: concept and vision
Discussion

Coffee

16.30-16.50
MAURIZIO SCARPA
Centre for Rare Diseases at the Helios Dr. Horst Schmidt Kliniken, Wiesbaden, DE
The rare Metabolic Diseases European Reference Network (MetabERN) action
Discussion

17.00-17.20
MAURIZIO SCARPA
InNerMeD-I Network Project Coordinator and President of the B4B, IT
Inherited NeuroMetabolic Diseases Information Network (InNerMeD-I Network) as a tool for validated scientific information
Discussion

17.30 -18.30
General discussion on the organization of research activities driven by B4B in the MetabERN

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March 19th 2016

INDUSTRY AND EUROPEAN COLLABORATIONS

CHAIR DISCUSSANTS
DAVID WHITEMAN, USA
AND DAVID BEGLEY, UK

9.00-9.25
ANNE CHRISTIANSEN
Shire, USA
Histological changes associated with disease progression in the neurovascular unit of the MPSII mouse
Discussion

9.35-10.00
ANA CRISTINA PUGA
Sanofi Genzyme, FR
Crossing the Barrier and Meaning it: Evaluation of a novel Substrate Reduction Therapy in Gaucher Disease Type 3
Discussion

10.10-10.35
SANDRA ROJAS-CARO
Alexion Pharmaceuticals Inc, USA
Preclinical Results of Heparan Sulfate Content in the Brain following Intravenous SBC-103 administration in a Mucopolysaccharidosis IIIB Mouse Model and Initial, 24 week Results of Heparan Sulfate Levels in Cerebrospinal Fluid (CSF) and Serum in an Open Label, Phase I/II, First-in-Human Clinical Trial of Intravenous SBC-103 in Mucopolysaccharidosis IIIB patients
Discussion

Coffee

11.15-11.40
REINHARD GABATHULER
Bioasis Technologies Inc, CA
Administration of Fusion proteins incorporating MTfpep or MTf in a Lysosomal Enzyme (I2S) delivers a Therapeutical Concentration of I2S to the CNS to treat MPS II (Hunter Syndrome)
Discussion

11.50-12.15
TEMITAYO AJAYI
BioMarin Pharmaceutical Inc, USA
CLN2 study with BMN 190 Phase 1/2 Study with Cerliponase Alfa
Discussion

LUNCH AND FAREWELL
To the next 2017 meeting

NOTES

MECHANISMS OF ENDOSOME BIOGENESIS, FUSION AND SIGNALLING

MARINO ZERIAL
Max Planck Institute of Molecular Cell Biology and Genetics, MPI-CBG, Dresden, Germany

A major challenge in biological research is to integrate multiple levels of complexity, hierarchically ordered from molecules to molecular assemblies, to organelles, cells, tissues, organs and entire organisms. We have previously demonstrated that the small GTPase Rab5 is a master regulator of early endosome biogenesis. Rab5 is both necessary and sufficient, together with SNAREs, for the biogenesis of fusion-competent early endosomes *in vitro* for the entire endo-lysosomal pathway *in vivo*. Rab5 regulates the specificity and directionality of endosome fusion via the recruitment of tethering effectors that lead membranes to dock and fuse. One of the outstanding questions is which are the molecular and physical mechanisms by which membranes are brought together to dock after capture by a tether. Our studies on the Rab5 effector EEA1 revealed novel biophysical properties of this protein that shed light into this question and have important implications for intracellular membrane transport in general. At the cellular scale, we explored the design principles of the early endosomal network, which is shaped as a *funnel* by a balance of fusion, fission and endosomal conversion. We found that the endosomal network has a surprising role in determining the amplitude, lifetime and robustness of the signalling response.

REDUCING ATTRITION RATE: IMPLEMENTATION OF HUMAN *IN VITRO* BBB MODELS INTO THE FLOW OF STANDARD ASSAYS USED IN DRUG DISCOVERY

ROMÉO CECCHELLI, AURORE DROLEZ, FABIEN GOSSELET, CAROLINE MYSIOREK, MARIE-PIERRE DEHOUCK
Blood Brain Barrrier laboratory, Université d'Artois EA 2465, 62307 Lens, France

Central Nervous System (CNS) disorders are some of the most prevalent, devastating and yet poorly treated illnesses that constitute one of the key areas for drug development. It is interesting to note that CNS, a therapeutic area with very high attrition rate, is also an area which animal models are not very predictive of the human pathophysiology. So it is important that the mindset of reducing attrition in development should be in place from the earliest stage of discovery. Recommendations for CNS drug discovery and development are i; do not rely solely on recombinant systems but develop in vitro systems that are physiologically relevant ii, pay careful attention to the pharmacokinetics and Blood-Brain Barrier (BBB) penetration properties of compounds and ensure adequate exposure of drugs to the CNS targets (Pangalos et al; 2007)
Due to inter-species differences at both the neural and BBB levels, cell-based drug screening and testing methods that use transformed cell lines or primary animal cells are not adapted. However recent advances in the use of **human** stem cells in BBB modelling open opportunities to develop improved human in vitro physiological models capable of simulating more realistically in vivo-like responses to neurotoxicants or neurotropics.
The talk will review recent advances in the generation and use of human blood brain barrier models for drug discovery and in the oncology area and would highlight the differences between human and non-human BBB models. Recent studies demonstrated BBB differences among different species being the most important related to the expression of P-gp, multidrug resistance-associated proteins and transporters. Therefore, human BBB models may be a more reliable platform for initial drug screening, prior to *in vivo* studies. After selection and ranking of the hits *in vitro*, they can be tested against *in vitro* rodent models, which are a bridge between human *in vitro* models and rodent *in vivo* models. The combination of both tests might help the reduction of cost in drug development process and failures in clinical studies.

REVISITING ASPARTYLGLUCOSAMINURIA: CHARACTERIZATION OF STRUCTURAL CONSEQUENCES OF NOVEL AND OLD MUTATIONS REVEALS PROSPECTS FOR THERAPY FOR AGU

ANTJE BANNING, CHRISTINA GÜLEC, JAN F. KÖNIG, RITVA TIKKANEN*
Institute of Biochemistry, Medical Faculty, University of Giessen, Friedrichstrasse 24, D-35392 Giessen, Germany
** Presenting author*

Aspartylglucosaminuria (AGU) is a recessive glycoprotein storage disorder that is caused by mutations in the gene for the lysosomal enzyme aspartylglucosaminidase (AGA). This enzyme is involved in glycoprotein degradation and cleaves the bond between asparagine and the carbohydrate. Missing AGA activity results in a progressive mental retardation of AGU patients from early childhood on, but the life expectancy is not severely compromised. AGU is most common in Finland, but an increasing number of AGU cases have recently been diagnosed elsewhere in the world. AGA is a heterotetrameric enzyme $(\alpha\beta)2$ that is synthesized as a catalytically inactive, single-chain precursor molecule that is autocatalytically cleaved into the active form. Almost all AGU mutations reside outside of the active site of AGA, but they impair the proteolytic processing of the AGA precursor into the subunits. The structural consequences of only few AGU mutations have been dissected. The most common disease mutation Cys163Ser, also called AGUFin, results in a loss of a disulfide bond, impairing the folding of AGA. However, in the case of many other mutations, the structural defects are not that evident. We have here characterized the consequences of some newly-identified AGU mutations and show that these mutations cause a local misfolding of AGA, which is sufficient to impair the processing into the active form. However, these mutant enzymes principally retain their capacity to be activated and are localized in lysosomes. Dimerization of the mutant enzyme precursor with the wildtype AGA results in enhanced normal processing of the mutant enzyme. Our data thus suggest that these AGU mutations cause a local misfolding which can be reverted by providing a normally folded "folding aid". However, the correct folding of the mutant AGA may also be obtained by means of pharmacological chaperones (PCs). We will present data with newly identified PCs showing that enzymes carrying various AGU mutations can indeed be activated in patient cells by these compounds. This opens novel prospects for chaperone-mediated therapy for AGU.

NATURAL HISTORY OF NIEMANN-PICK DISEASE TYPE C IN THE UK

JACQUELINE IMRIE
NPUK

This Presentation will give an overview of a recently published document, *Observational cohort study of the natural history of Niemann-pick disease type C in the UK: a 5-year update from the UK clinical database. Imrie et al 2015 BMC Neurology.*

Background

Niemann-Pick disease Type C (NPC) is a rare neurovisceral lysosomal storage disorder with varying age of onset, severity being related to age of onset of neurological symptoms. Symptoms are progressive and disabling invariably to early death

Methods

This was an observational study of all patients with NPC known by the Clinical Nurse Specialist based in Manchester. Patients were stratified according to internationally accepted age at neurological onset categories. Data on age of onset of symptoms, clinical signs and genetic data was summarised.

Results

A total of 146 patients were included, taken from database information between 1999 and the end of 2011. 72 of these patients were still alive at the close of the study. Most patients had NPC1 with only 2 having NPC 2. 8 had early infantile onset; 51 late-infantile; 42 juvenile and 25 adolescent/adult. 6 patients had an early, non-neurological form and 14 patients did not have any neurological signs at last follow up.

51 patients had received Miglustat therapy with the mean overall treatment duration being 2.6y.

Conclusions

Although the UK cohort is the largest national NPC group to be reported to date, important studies have been published from the Chezch republic and other countries. All this data is a valuable resource. The International Niemann-Pick Disease Registry will hopefully capture more patients from all over the World to give an even bigger overview of the true natural history of NPC.

SUCCESSFUL TREATMENT OF KRABBE DISEASE - THE HOLY GRAIL AMONG HEREDITARY LEUKODYSTROPHIES

M BEGOÑA CACHÓN-GONZÁLEZ
University of Cambridge, Department of Medicine, Cambridge, UK

Krabbe disease or Globoid cell leukodystrophy (GCL), inherited as an autosomal recessive trait, is caused by dysfunction of the lysosomal enzyme β-galactocerebrosidase (GALC) or the sphingolipid activator saposin A. Saposin A mediates the interaction between GALC and substrates - facilitating the hydrolysis of lipids such as myelin-rich galactocerebroside and cognate galactosylsphingosine. Disease-causing mutations in genes GALC and PSAP disrupt the normal turnover of these sphingolipids, with devastating consequences for the central and peripheral nervous systems. Paradoxically, accumulation of galactocerebroside is not apparent, but galactosylsphingosine, barely detectable in healthy nervous tissue, is overrepresented in GCL. Pathological features of the disease include reduced peripheral and central myelin, axonal degeneration, gliosis and conspicuous presence of globoid cells.

Onset of clinical manifestations in the more common infantile form occur at 3-6 months of age, hyperesthesia and irritability is soon followed by hypertonicity and seizures, with death around the second year of life. Late-onset patients have a less stereotypical disease course, but often present with muscle weakness, blindness and cognitive regression. Treatment by bone marrow and umbilical cord-blood cell transplantation, although not curative, has shown promise in a small number of pre-symptomatic infants and older patients - palliative care being the norm.

Authentic animal models of the condition - mice, dogs and non-human primates - are available for experimental interrogation of underlying mechanisms of disease and for testing new therapies. Bone marrow, neural and mesenchymal stem cell transplantation, substrate reduction and pharmacological chaperone therapy, gene and enzyme replacement therapy, genetic reduction of macrophage/microglia numbers and combinations of these stratagems have been tested principally in the Twitcher mouse. Best outcomes resulting from these interventions showed delayed disease onset and significant extension of life.

Although there is currently no definitive treatment to cure this unforgiving disease, there is however undisputable evidence that proves the condition not to be refractory to treatment. It is anticipated that a combination of treatment modalities will be necessary for complete rescue. Renewed research emphasis aimed at deepening our understanding of the molecular mechanisms that lead to disease, beyond the primary defect, would accelerate our ultimate goal. My presentation will include our contribution to these endeavours.

THE CONTRIBUTION OF MUTANT GBA TO THE DEVELOPMENT OF PARKINSON'S DISEASE IN *DROSOPHILA*

GALI MAOR[1], OLGA KRIVOTUK[1], OR CABASSO[1], ORLY SELA[1], DANIEL SEGAL[2, 3] AND MIA HOROWITZ[1, 3]

[1] Dept of Cell Research and Immunology, [2] Department of Molecular Microbiology and Biotechnology and [3] Sagol Interdisciplinary School of Neurosciences, Tel Aviv University, Ramat Aviv, 69978, Israel.

Gaucher disease (GD), an autosomal recessive disease, results from mutations in the acid β-glucocerebrosidase (GCase) encoding gene, GBA, which leads to lysosomal accumulation of glucosylceramides. GD patients and carriers of GD mutations have a significantly higher propensity to develop Parkinson's disease (PD) in comparison to the non-GD population. We have previously shown that in cells derived from patients of GD and carriers of GD mutations, mutant GCase variants, retained in the ER, lead to ER stress and to activation of the ER stress response, known as the unfolded protein response (UPR).

In the present study we used the fruit fly *Drosophila melanogaster* to confirm that development of PD in carriers of GD mutations results from the presence of mutant GBA alleles. *Drosophila* has two GBA orthologs (CG31148 and CG31414), each of which has a minos insertion, which creates C-terminal deletion in the encoded GCase. Flies double heterozygous for the two endogenous mutant GBA orthologs presented UPR and developed Parkinsonian signs, manifested by death of dopaminergic cells, defective locomotion and a shorter life span. We also established transgenic flies carrying either the normal or the mutant N370S, L444P and the 84GG variants of human GCase. UPR activation and development of Parkinsonian signs could be recapitulated in flies overexpressing these three mutant variants of GCase alleles.

UPR and Parkinsonian signs could be partially rescued by growing the double heterozygous flies, or flies containing the N370S or the L444P human mutant GCase variants, in the presence of the pharmacological chaperone ambroxol, which binds and removes mutant GCase from the ER. However flies expressing the 84GG mutant variant, which does not express mature GCase, did not exhibit rescue by ambroxol.

Our results strongly suggest that the presence of a mutant GCase in dopaminergic cells leads to ER stress and to their death, and contributes to development of Parkinson's disease.

MAKING THE CASE FOR AMBROXOL FOR TYPE 3 GAUCHER DISEASE: A 15-YEAR LONG SAGA WITH A NEW TWIST FOR USE IN PARKINSON

ARI ZIMRAN, DEBORAH ELSTEIN
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Five years ago in this forum we presented the results of a pilot study using oral ambroxol (Zimran et al. Blood Cells Mol Dis. 2013 Feb;50(2):134-7) as a pharmacological chaperone (Maegawa et al. J Biol Chem. 2009 Aug 28;284(35):23502-16) in patients with type 1 Gaucher disease (GD); we monitored hematological and visceral signs. Our conclusions highlighted very specific action points should we intend to employ this modality in GD: [1] it was safe; [2] patients were compliant with a three-times daily regimen; and, importantly, that [3] the dosage used which was the highest approved for over-the-counter (OTC) indications, was insufficient for heavier patients to benefit and hence a higher dosage and one based on body weight would be required. However, the underlying premise of the study was that eventually this drug would be appropriate for neuronopathic GD (Castilla et al. J Med Chem. 2012;55(15):6857-65). In the intervening years, the Japanese group of Ohno and Narita, based on important pre-clinical investigations by others as well (Luan et al, Brain Dev. 2013;35(4):317-22; Bendikov-Bar et al. Blood Cells Mol Dis. 2013;50(2):141-5.Narita et al. Ann Clin Transl Neurol. 2014;1(2):135-40; Castill et al. Eur J Med Chem. 2015;90:258-66), has shown that clinically relevant change in neurological signs of type 3 GD including myoclonic seizures and even supranuclear horizontal gaze palsy, was effected by appropriate doses of ambroxol. In addition, patients and some treating physicians have been galvanized to treat the signs of Parkinson disease (McNeill et al. Brain. 2014;137(Pt 5):1481-95) that are seen in patients with GD. We believe that use of ambroxol for carriers of GD mutations with Parkinson disease also seems plausible as a therapeutic option.

Therefore, we have begun to think about an ambroxol registry to monitor safety and efficacy by reaching out to treating physicians prescribing ambroxol; but this has a retrospective rather than proactive function. We have been talking about ambroxol for nearly 15 years in the hope of acquiring the patented rights to this drug and employing it in clinical trials with patients with type 3 (and possibly 3c) and for those GD patients and carriers with Parkinson disease. We believe that too much time has elapsed in discussions and we are hopeful that B4B can make the case in the European Union for funding of a clinical trial at minimum for young adults with type 3 and those patients and carriers with GD and Parkinson disease.

C-TERMINAL FRAGMENTS OF AMYLOID PRECURSOR PROTEIN (APP): FINGERPRINTS OF SUBCELLULAR STORAGE IN NEURODEGENERATIVE DISEASES

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An early hallmark of neurodegeneration in Alzheimer's disease (AD) is the dysfunctional appearance of organelles belonging to the endosomal-autophagic-lysosomal (EAL) system. This pathology may arise from an impaired clearance of endosomes and autophagosomes by lysosomes (lysosomal flux) (Boland et al, 2008). Possible causes of EAL dysfunctional in the AD brain may be due to (i) inefficient lysosomal enzyme activity (primary flux impairment) or (ii) inefficient cargo delivery to lysosomes (secondary flux impairment). In this study, we set out to determine if either/both/none of these factors may contribute to AD pathology. To achieve this interpretation, we developed a biomarker approach based on specific metabolites of amyloid precursor protein (APP), which were found to delineate between primary and secondary flux impairments.

Amyloid precursor protein (APP) is one of a number aggregate-prone proteins whose metabolism becomes altered in neurodegenerative diseases. APP is predominantly cleaved by α- or β-secretase at the plasma membrane / early endosomes, forming transitory metabolites known as APP C-terminal fragments (APP-CTFs). APP-CTFs can subsequently undergo additional cleavage by γ-secretase, to produce either the non-aggregate-prone p3 peptide (α-secretase-dependent) or the aggregate-prone amyloid-β-protein (Aβ) (β-secretase-dependent). Although the majority of APP is metabolised by canonical α-, β-, γ-processing, a significant proportion of APP and its metabolites are directed to lysosomes for internal catabolism. Considering lysosomes catabolise APP-CTFs containing the Aβ region, and free Aβ itself, the integrity of the EAL system serves an important anti-amyloidogenic and neuroprotective function.

We have previously described the abundant accumulation of autophagic vacuoles (AVs) and APP-CTFs in brain tissue of three different lysosomal storage disease mouse models: Niemann Pick Type C1, Sandhoff disease and GM1 Gangliosidosis (Boland et al, 2010). Detailed analysis of the specific APP-CTFs that accumulate in these lysosomal storage diseases revealed the presence of two non-canonical CTFs (CTF-6 and -7) of lower molecular weight than the five canonical CTFs generated by α- and β-cleavage. The presence of CTF-6 and -7 was minimal/absent in healthy rat primary cortical neurons and wild type mouse brains, but were expressed in cultured neurons treated with

cathepsin B and L inhibitors (a primary storage phenotype). However, neurons treated with the class II amphiphile, U18666A, which impairs endosomal-lysosomal trafficking (secondary storage phenotype), only expressed five canonical CTFs, even in the presence of cathepsin B and L inhibitors. Addition of the lysosomal rupturing agent, GPN, to cultured neurons pre-treated with cathepsin L inhibitors, showed a rapid depletion of CTF-6 and -7, providing further evidence that CTF-6 and -7 are generated and degraded in lysosomes.

Recent analysis of APP-CTF profiles in post-mortem AD and age-matched control brain tissue (medial temporal gyrus), resulted in the successful detection of five canonical CTFs, however, the expression of CTF-6 and -7 was minimally detected in both cohorts. In addition, levels of active cathepsin D, a marker of lysosomal acidification, were also found to be unaltered between groups. These findings indicate that unlike classical lysosomal storage diseases where progressive neuropathology is associated with an abundant accumulation of AVs and APP-CTFs in degenerating brain tissue, expression profiles of APP-CTFs in the AD brain indicate that the absence of primary lysosomal storage. By addressing fundamental reasons why neuron function becomes impaired in lysosomal storage diseases and AD, this research aims to improve our understanding of their pathogeneses and identify new diagnostic and therapeutic strategies.

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THE NATURE OF GLIAL DYSFUNCTION AND ITS IMPACT UPON NEURONS VARIES BETWEEN FORMS OF NCL

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In all forms of Neuronal Ceroid Lipofuscinosis (NCLs, or Batten disease), localized glial activation appears to be an early event that accurately predicts where neurons subsequently die. This raises the question whether these events are linked, and what the glial contribution to pathogenesis might be. To begin answering these questions we have grown glial and neuronal monocultures, and a variety of different co-cultures derived from mouse models of the three major forms of NCL (Cln1, Cln2, and Cln3 diseases). This has allowed us to explore whether the biology of glia, which would normally express the deficient proteins, is compromised and if this has any subsequent effects upon neurons.

In the most prevalent form of NCL, Cln3 disease (Juvenile NCL, JNCL), we found basic defects in both Cln3 deficient microglia and astrocytes. Mutant microglia failed to transform morphologically and displayed an altered protein secretion profile upon stimulation. These defects were far more pronounced in astrocytes, in which cytoskeletal abnormalities, impaired calcium signalling and reduced glutamate clearance were observed. Furthermore, when grown in a co-culture system, Cln3 deficient glia were shown to negatively impact the health of both Cln3 deficient and wildtype neurons, with mutant neurons being the most severely affected.

In Cln1 disease, we have identified a range of different glial phenotypes, with both mutant astrocytes and microglia displaying activated morphologies under basal conditions, but displaying relatively normal responses to stimulation and a no cytoskeletal abnormalities. However, unlike Cln3 deficient cultures, we found a pronounced defect in Cln1 deficient astrocyte survival, and in the phagocytic ability of Cln1 microglia. As with Cln3 deficient glia, we also found a pronounced deleterious influence of Cln1 deficient glia upon neuronal morphology and survival.

In marked contrast, Cln2 deficient astrocytes or microglia displayed no obvious phenotypes, when grown under either basal or stimulated conditions. Instead we have found a range of neuronal phenotypes, distinct to those found for either *Cln1* or *Cln2* deficient neurons.

Taken together these data reveal that the extent and nature of glial dysfunction appears to vary markedly between the three major forms of NCL. Cln2 disease appears to involve neurons to a greater extent than either Cln1 or Cln3 disease, both of which show more pronounced glial defects which have a negative impact of this upon neuronal health. These data reveal fundamental differences between this group of disorders and raise the possibility that glia should also be considered as therapeutic targets in some forms of NCL.

MUCOPOLYSACCHARIDOSIS IIIA STORAGE SUBSTRATE DRIVES AN INNATE IMMUNE NEURO-INFLAMMATORY RESPONSE

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Mucopolysaccharidosis type IIIA (MPSIIIA) is a paediatric lysosomal storage disease characterised by mutations in the sulfo-glucosamine sulfo-hydrolase (SGSH) gene, resulting in reduced lysosomal SGSH enzyme activity. Subsequently, an accumulation of highly sulphated, partially degraded heparan sulphate oligosaccharides occurs in lysosomes and the extracellular matrix with disease progression, alongside secondary accumulation of GM gangliosides, cholesterol and amyloid beta. MPSIIIA patients develop behavioural disturbances and progressively worsening cognitive deficits, ultimately leading to dementia and premature death, a possible consequence of neuro-inflammation. Brains from MPSIIIA mice demonstrate markedly increased astrocytosis and microgliosis, particularly in the cortex[1]. This, coupled with up-regulation of IL-1α, IL-1β and TNFα, suggests the development of a pro-inflammatory environment in MPSIIIA. However, the molecular mechanisms responsible for neuro-inflammation in MPSIIIA remain unclear. This project aims to understand how HS and secondary storage substrates affect the CNS and neuro-inflammatory pathways.

Here we show that glycosaminoglycans (GAGs) isolated from MPSIIIA mice induce pro-inflammatory TNFα, IL-6, IL-1α and IL-1β responses when applied to a primary mixed glial culture, where normalised levels of WT GAGs did not elicit a response. The data also shows that qualitative alterations, rather than amount, in MPSIIIA GAGs are responsible for inflammatory responses. MPSIIIA GAGs act as an inflammatory priming stimulus via toll-like receptor 4 (TLR4) as inhibition of the intracellular domain of TLR4 completely abrogated the inflammatory response ($p \leq 0.001$). MPSIIIA GAGs did not initiate IL-1 dependent signalling alone in this in vitro model; indeed recombinant IL-1 receptor antagonist (IL-1Ra) did not reduce the inflammatory response. Secondary stimulation with NLRP3 inflammasome activators such as ATP or secondary storage substrates accumulated in MPSIIIA (monohydrate cholesterol crystals or amyloid beta oligomers), induced the release of intracellular IL-1β associated with MPSIIIA GAG priming ($p \leq 0.001$).

In vitro data suggests that MPSIIIA neuro-inflammation may be dependent on IL-1, and driven by primary and secondary storage substrates. We are currently performing in vivo studies to confirm whether the MPSIIIA neuro-inflammatory response acts through IL-1 dependent mechanisms, and whether modulation of the innate immune response will slow disease progression.

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TIGHT JUNCTIONS IN THE BLOOD-BRAIN BARRIER UNDER STROKE AND CLAUDIN MIMETICS

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The exact role of the blood-brain barrier (BBB) in stroke as well as specific modulation of BBB tightness is unknown. The barrier-forming endothelium paracellularly sealed by tight junction (TJ) proteins protects the brain homeostasis against peripheral disturbances and ensures metabolite exchange. On the other hand, the barrier limits brain delivery of many pharmaceuticals. Sealing of the BBB is mainly caused by claudins (Cldns) 5, 3 and 1. We therefore aim at elucidation of structure and function of cerebral TJs after stroke and claudin peptidomimetics.

Mice were analysed for BBB permeability (small/large molecules), expression (mRNA/protein amount) and morphology (electron microscopy, immunohistochemistry) of the TJs in vitro after hypoxia and in vivo after transient middle cerebral artery occlusion (MCAO; staining/quantification of stroke/oedema). New findings are that Cldn3 tightened the BBB for small molecules, limited endothelial endocytosis and transcytosis of proteins, complemented TJ morphology, prevented inflammation-related processes, and regulated Cldn1, Cldn5 and occludin. After acute hypoxia of isolated mouse brain capillaries, the BBB specific TJ marker Cldn5 remained unaffected in the presence of Cldn3; in its absence, Cldn5 declined at the TJs. In the postischemic infarction process, Cldn3 accounted for increased infarct volume due to increased swelling of the affected brain. In addition, claudin mimetics (peptides, small molecules) were designed. They caused permeability rise through cell culture models of cerebral barriers (bEnd, MDCK-Cldn5) and through the mouse BBB in vivo.

In summary, Cldns 5, 3 and 1 contribute to the intactness of the BBB under physiological and pathological conditions, protect the BBB in stroke but prevent detumescence of the injured area, hence worsening infarct outcome. Thus, modulation of Cldns tightening the BBB might help to improve recovery from stroke as well as drug delivery to the brain.

TIGHT JUNCTION PROTEIN EXPRESSION AND NEUROLOGICAL DISEASE

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Endothelial cells associated with the brain have properties distinct from endothelial cells in peripheral organs of the body. These cells are held together by tight junctions, which, as the name implies, form a tight seal limiting paracellular transport between adjacent cells. These tight junctions are comprised of a series of up to 30 proteins, interacting to provide a tight seal between adjacent endothelial cells lining the neural microvasculature. As well as regulating the exchange of ions and macromolecules between the blood and the delicate neural microenvironment, these highly specialized endothelial cells protect the brain by restricting the entry of potentially damaging blood-borne agents such as neurotoxic chemicals, antibodies, pathogens, immune cells and anaphylatoxins.

A number of genetic factors have identified susceptibility loci on chromosome 22 (22q11.21) that appear to segregate with neuro-developmental disorders such as schizophrenia. Located within this region is the gene claudin-5, a fundamental component of the tight junction between endothelial cells in the blood-brain barrier (BBB). Reduced levels of claudin-5 lead to increased permeability of the blood-brain barrier (BBB), which may be associated with some of the symptoms associated with psychosis. Here, we investigate the behavioural effects of claudin-5 suppression in mice. An adeno-associated virus (AAV-2/9) vector containing a doxycycline-inducible gene encoding shRNA targeting claudin-5 was stereotaxically injected either into the dorsal hippocampus (dHipp) or the medial prefrontal cortex (mPFC). Mice were allowed to recover for 7 days before 2 weeks of doxycycline treatment. Animals subsequently underwent a battery of behavioural tasks covering various aspects of normal behaviour: learning and memory (object recognition task; spatial navigation); anxiety (open field task); depression (forced swim task; splash test); social behaviour (social interaction task); motor co-ordination (neurological severity score; Rota-rod); spatial gating (paired-pulse inhibition) and observation of home cage behaviours. By comparing claudin-5 suppression in dHipp and mPFC across these tasks, it is possible to elucidate the effects of selectively modulating BBB permeability on normal behaviour in mice and in the development of a BBB-based model of psychosis.

THE CURRENT MODEL OF WHY HEMATOPOIETIC STEM CELL TRANSPLANTATION IS MORE POTENT IN HALTING THE INFLAMMATION IN X-LINKED ADRENOLEUKODYSTROPHY THAN IN HALTING THE DISEASE PROGRESSION IN METACHROMATIC LEUKODYSTROPHY

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In contrast to the lysosomal disorder Metachromatic leukodystrophy is X-linked adrenoleukodystrophy (X-ALD) caused by mutations in the ABCD1 gene encoding a peroxiosmal ABC transporter also named ABCD1. In about 60% of male X-ALD patients, either in childhood or in adulthood, a devastating, rapidly progressive form of cerebral inflammatory demyelination occurs. Allogenic hematopoietic stem cell transplantation and autologous stem cell-based gene therapy can arrest the inflammatory demyelinating process with a typical delay of 12-18 months. In contrast to the lysosomal enzymes such as arylsulfatase A, the peroxisomal transporter ABCD1 is not segregated and transmitted to other cells. As the extent of the metabolic defect in the main immune cells in X-ALD was unknown, we explored their phenotypes. Interestingly, the monocyte showed the severest biochemical phenotype with a sixfold accumulation of C26:0 and a striking 70% reduction in peroxisomal β-oxidation activity. In contrast, VLCFA metabolism was close to control values in B cells and T cells. Based on these results, we propose that in X-ALD the halt of inflammation after allogeneic hematopoietic stem cell transplantation relies particularly on the replacement of the monocyte lineage. Thus, totally different molecular mechanisms might explain why the peroxisomal inflammatory disorder presents with a more effective therapeutic benefit than the lysosomal disorder metachomatic leukodystrophy.

UPTAKE MECHANISM OF APOE-MODIFIED NANOPARTICLES

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The blood-brain barrier (BBB) is one of the most important and impermeable physiological barriers in the organism. It protects our delicate central nervous system homeostasis by shielding off toxic metabolites, extraneous substances and attacks of pathogens and represents an insurmountable obstacle for most drugs, thus obstructing an effective treatment of many brain diseases.Therefore, a number of different strategies have been employed during the past years to overcome this barrier. One of them is the fast-emerging field of nanotechnology. It offers the possibility to transport drugs over the BBB by packing them into surface-modified nanoparticles (NP) 1. Especially apolipoprotein E (ApoE) appears to play a major role in the nanoparticle-mediated drug transport across the BBB. Further studies verified a clear correlation between the ApoE adsorption and the BBB passage 2, 3. Therefore, it was predicted that these NP resemble endogenously circulating lipoproteins and are taken up by a receptor-mediated pathway by the brain endothelial cells, which express the respective receptors. In our studies, we could verify this uptake mechanism of the ApoE-modified NP and identify the low density lipoprotein receptor related protein 1 (LRP1) as the corresponding receptor. Different co-incubation experiments with RAP, a protein that blocks all binding sites on most receptors of the LDLR family, and soluble fragments of the LRP1, which contained the main LRP1 binding domains II and IV were done.
This knowledge of the uptake mechanism of ApoE-modified NP into the brain enables future developments to rationally create very specific and effective carriers.
As an example, we focus on transporting NP loaded with an anti-Alzheimer´s disease (AD) drug to the brain: Our data suggest that embedding flurbiprofen (a drug that failed in clinical trials due to its low permeability to the brain) in poly(lactic acid) NP enables crossing of an advanced in vitro BBB model. In fact, we observed an amyloid ß42 (the peptide proposes to cause AD) – lowering effect in the brain-compartment after application of flurbiprofen loaded NP to the blood-compartment. With regard to promising pilot binding experiments, we are confident that this outcome can be further optimized by coupling ApoE to the NP surface.

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NANOPARTICLE-BOUND DOXORUBICIN FOR CHEMOTHERAPY OF GLIOBLASTOMA: OVERCOMING BARRIERS

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The blood-brain barrier (BBB) restricts penetration of the drugs to the brain thus limiting the efficacy of chemotherapy for many brain diseases, the most detrimental of which is probably glioblastoma. The strategies employed presently for delivery of the cytotoxic agents to the brain are invasive and, in spite of many efforts to solve the problem, there is still a huge unmet need for the technology that enables safe access to the brain.

The concept of brain delivery by nanoparticles emerged in the nineties from a surprising finding that the drugs unable to efficiently penetrate through the BBB, produced the CNS effects after binding to the biodegradable poly(butyl cyanoacrylate) nanoparticles (PBCA NP) simply coated with certain pharmaceutical surfactants. However, it took more than 20 years until, through certain skepticism, the nanoparticles became recognized as promising delivery systems for drugs that normally cannot easily circumvent the BBB.

Surfactant coating appears to be the key factor of this technology. This coating promotes the adsorption of blood plasma apolipoproteins (i.e. A-I or E) that mediate the NP interaction with the brain endothelial cells followed by either endocytosis, or transcytosis into the brain. Thus, according to our present understanding, the poloxamer 188-coated PLGA NP represent a biomimetic delivery system that follows the route of the naturally occurring lipoprotein particles and enters the brain via endogenous mechanisms. This system in a way is also self-assembling because, being introduced into the blood stream, the nanoparticles spontaneously acquire a suitable vector ligand.

A considerable anti-tumour effect of doxorubicin (DOX) bound to polysorbate 80- or poloxamer 188-coated PBCA NPs against an intracranially implanted 101.8 rat glioblastoma so-far was the most important result enabled by this technology. The conventional formulation of DOX was only marginally effective in this model because the brain uptake of free DOX is very low.

Later we were able to demonstrate that this technology also can be applied to NPs made of poly(lactide-co-glycolide) (PLGA) which is known to be safe and biocompatible. Intravenous administration of DOX bound to the poloxamer 188-coated PLGA NPs (Dox-PLGA) consistently enabled a considerable growth inhibition of 101.8 glioblastoma and long-term remission in >20% animals. Microscopic studies revealed the effective penetration of the nanoparticles into the brain and a preferential accumulation in the tumour.

Extensive preclinical studies confirmed the efficacy of Dox-PLGA and demonstrated its favorable toxicologi-

cal profile. The most important finding was the reduction of DÖX cardiotoxicity that could be explained by the altered biodistribution of the nanoparticle-bound drug.
The above technology was successfully optimized, scaled-up, and transferred to industry.
A Phase I dose escalation study of the nanoparticle-bound doxorubicin in patients with advanced solid tumours (including GBM) is currently on-going in Russia. The drug is well tolerated and does not cause any dose-limiting adverse effects at the dose levels studied so-far.

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SAFELY ENHANCING BRAIN DRUG DELIVERY USING THE G-TECHNOLOGY IN CLINICAL PROGRAMS TARGETING BRAIN CANCER AND NEURO-INFLAMMATION

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Many CNS diseases remain insufficiently treated due to poor drug efficiency and/or insufficient concentrations of the drug in relevant brain tissues. While several promising drug candidates are available for various CNS disorders there is a need to increase their ability to effectively cross the blood-brain barrier (BBB) to make them useful in clinical practice. The endogenous tripeptide glutathione (GSH) is found at high levels in the brain and is actively transported across the blood-brain barrier. Therefore, glutathione PEGylated liposomes (G-Technology®) were developed to mediate safe targeting and enhanced delivery of encapsulated drugs to the brain.
2-BBB's lead product, glutathione PEGylated liposomal doxorubicin (2B3-101), is based on PEGylated liposomal doxorubicin (Doxil®/ Caelyx®) and was developed as brain-targeted chemotherapy. In preclinical studies, 2B3-101 showed a 5-fold enhanced delivery of doxorubicin to the brain compared to Doxil/Caelyx, and an improved survival of mice with experimental glioblastoma. Furthermore, the GLP toxicity studies showed no major differences between 2B3-101 and Doxil; no cardiotoxicity and neurotoxicity was observed. A clinical trial was designed to determine the safety, tolerability and pharmacokinetics of 2B3-101 in patients with solid tumors and brain metastases or recurrent malignant glioma. The dose-escalation phase I part of the study has been completed, and the phase IIa part of the study to determine preliminary antitumor efficacy at the maximum tolerated dose has completed reqruitment. Efficacy and saftey profile were found to be encouraging and warrant next stage in development.
2-BBB's second product in development is glutathione PEGylated liposomal methylprednisolone (2B3-201) for the treatment of patients with acute and chronic neuroinflammation. Methylprednisolone (MP) has beneficial therapeutic properties, yet its use is limited by several (severe) acute and chronic side effects or invasive local delivery routes. Systemic administrations of 2B3-201 have recently resulted in superior efficacy and reduced side effects compared to the free MP in several rodent models with neuroinflammation. 2B3-201 was subsequently investigated in a pharmacokinetic and biodistribution study and compared to free MP, showing a dramatically enhanced plasma circulation half life, and higher sustained levels of 2B3-201 in brain and spinal cord. Furthermore, therapeutic doses of 2B3-201 did not result in psychotic-like behavioral effects in rats, as were clearly demonstrated by free MP. Also, repeated weekly administrations of 2B3-201 were well tolerated in rats, while the same weekly doses of free MP were causing side effects. A phase I study in healthy volunteers was completed, addressing safety, tolerability and pharmacokinetics, and markers for pharmacological proof-of-concept.
Collectively, the mechanistic in vitro and in vivo preclinical and clinical studies performed to date have demonstrated that glutathione PEGylated liposomes (G-Technology®) offer a promising platform that could be used to safely enhance the delivery of drugs to the brain.

INTRA-CEREBRAL ADMINISTRATION OF AAV VECTOR CONTAINING THE HUMAN ALPHA-N-ACETYLGLUCOSAMINIDASE CDNA IN CHILDREN WITH SANFILIPPO TYPE B (MPSIIIB) SYNDROME: RESULTS AT 12 MONTHS OF A PHASE I/II TRIAL

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Four children with MPSIIIB mutated in the NAGLU gene received intracerebral deposits of highly purified rAAV2/5-hNAGLU vector particles produced by Sf9 insect cells engineered with baculovirus vectors (manufactured by Uniqure, NL). At the time of treatment, the youngest patient (20 months) had normal cognitive evaluation, two patients (26 and 30 months) were slightly below normal, and the oldest patient (53 months) was in the mild delay range. None of the children had autistic behaviour. The AAV vector dose of 4x1012 viral genomes/patient in 60µl was delivered over 2 hours at 16 sites in the cerebral and cerebellum white matter. Gene therapy was combined with immunosuppression (mycophenolate mofetil 8 weeks, tacrolimus, long-term) to prevent immune rejection. Low titer vector was detected in blood (≤2x103vg/µg DNA) and urine for 48hours post-deposition. Safety data collected over the one-year follow-up showed good tolerance. We observed no reactive inflammation on brain images, no adverse events related to product or procedure, no increase in number of infectious events, no sign of toxicity related to immunosuppressive drugs (except a short period of high transaminases in one child). Data on efficacy will be presented. Available preliminary results indicate release of catalytically active NAGLU in CSF, appearance of NAGLU-responsive T-lymphocytes in blood, normal brain development without atrophy at sequential MRIs, cognitive progression after one year, as measured through a large set of complementary neuropsychological testing.

INTRAVENOUSLY ADMINISTRATED ENZYME REPLACEMENT THERAPY IN THE MPSIIIA MALE MOUSE - REVERSAL OF LYSOSOMAL STORAGE AND INFLAMMATION IN THE BRAIN WITH GLYCAN MODIFIED SULFAMIDASE

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Several treatment options have been proven efficacious in the mouse model of mucopolysaccharidosis type IIIA (MPSIIIA), including intra-CSF administration of recombinant sulfamidase, intra-CSF AAV9 mediated gene therapy and lentiviral hematopoietic stem cell gene therapy. In contrast to these treatments, enzyme replacement therapy with intravenously administrated sulfamidase does not reduce CNS storage of heparan sulfate, the primary cause of the disease. In an attempt to improve the distribution of sulfamidase to the CNS, we investigated different protocols for glycan modification of sulfamidase. Conditions were found that generated a sulfamidase with retained stability and catalytic activity but with significantly reduced uptake in peripheral tissues. Distribution to the brain of the glycan modified sulfamidase could be demonstrated after intravenous administration. Efficacy studies in MPSIIIA male mice showed that weekly i.v. injections of the glycan modified sulfamidase reduced lysosomal storage in the brain as judged from quantitation of biomarkers for heparan sulfate storage, lysosomal LIMP-2 staining and presence of autofluorescent lysosomal deposits (lipofucin). Furthermore, a reduction in the number of activated microglia in cortex, hippocampus and cerebellum suggest that the treatment had a significant impact on CNS inflammation. Using an antihistamin pre-treatment protocol, the treatment was well tolerated. ADA was seen in some individuals and titers were, after an initial peek at 3-4 weeks of treatment, declining over time. This study indicates that systemically administrated enzyme replacement could be a treatment option to consider for amelioration of the neurological pathology in MPSIIIA.

DELINEATION OF NEURONAL PATHOLOGICAL EVENTS IN A CHEMICALLY-INDUCED MOUSE MODEL OF GAUCHER DISEASE

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Great interest has been shown in understanding the pathology of Gaucher disease (GD) due to the recently-discovered genetic relationship with Parkinson's disease. For such studies, suitable animal models of GD are required. Chemical induction of GD by inhibition of acid β-glucosidase (GCase) using the irreversible inhibitor, conduritol-B-epoxide (CBE), is particularly attractive, although few systematic studies examining the effect of CBE on development of symptoms associated with neurological forms of GD have been performed. We now demonstrate a correlation between the amount of CBE injected into mice and levels of accumulation of the GD substrates. Gene array analysis shows a remarkable similarly in the gene expression profile of CBE-treated mice and a genetic GD mouse model, the Gbaflox/flox;nestin-Cre mouse. Using this model, we also delineate the pathological processes in lysosomes and as a result the mechanism by which the neurons die. Together, our data demonstrate that CBE injection is a rapid and relatively easy way to induce symptoms typical of neuronal forms of GD and that injection of mice with CBE provides clues to the mechanisms by which GlcCer accumulation leads to neuronal pathology. These results will be of significance in assessing the efficacy of new therapies, as well as in improving the delivery of existing treatments and in suggesting new therapeutic approaches.

CHRONIC ADMINISTRATION OF AN HDAC INHIBITOR TREATS BOTH NEUROLOGICAL AND SYSTEMIC NIEMANN-PICK TYPE C DISEASE IN A MOUSE MODEL

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Histone deacetylase inhibitors (HDACi) are approved for treating rare cancers and are of interest as potential therapies for neurodegenerative disorders. We evaluated a triple combination formulation (TCF) comprising the pan-HDACi vorinostat, the caging agent 2-hydroxypropyl-b-cyclodextrin (HPBCD), and polyethylene glycol (PEG) for treating a mouse model (the Npc1nmf164 mouse) of Niemann-Pick type C (NPC) disease, a difficult-to-treat cerebellar disorder. Vorinostat alone showed activity in cultured primary cells derived from Npc1nmf164 mice but did not improve animal survival.However, low-dose, once-weekly intraperitoneal injections of the TCF containing vorinostat increased histone acetylation in the mouse brain, preserved neurites and Purkinje cells, delayed symptoms of neurodegeneration, and extended mouse life span from 4 to almost 9 months. We demonstrate that the TCF boosted the ability of HDACi to cross the blood-brain barrier and was not toxic even when used long term. Further, the TCF enabled dose reduction, which has been a major challenge in HDACi therapy. The TCF simultaneously treats neurodegenerative and systemic symptoms of Niemann-Pick type C disease and has potential for translation to other neurological disorders.

DAVID BEGLEY AND NANOPARTICLES

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The development of nanoparticles for the delivery of drugs across the blood-brain barrier and the involvement of David Begley in this development process and especially in the elucidation of the underlying mechanism will be highlighted from a historical perspective.

PHARMACOKINETICS OF BLOOD-BRAIN BARRIER TRANSPORT

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The ability to penetrate the blood-brain barrier (BBB) in order to gain access to the CNS is an essential prerequisite for drug candidates to be able to interfere with drug targets that reside within the brain. Addressing CNS penetration has therefore become an important element in today's CNS drug discovery. After a brief look at the flawed old concept of maximizing total brain levels, the presentation will focus on the key pharmacokinetic (PK) processes that control the unbound brain concentrations as the most relevant exposure parameter for pharmacological CNS activity (Reichel 2014, 2015).

There is now overwhelming evidence that the unbound brain concentrations represent the most predictive exposure parameter for the pharmacological activity of most CNS drugs (Hammarlund-Udenaes 2009). In contrast, neither total brain nor plasma concentrations can be reliably linked to pharmacodynamic (PD) and/or efficacy readouts and hence cannot be used as surrogate of the effect compartment to establish PK/PD relationships. For example, an increase in total brain levels generally does not result in an increase in the unbound drug concentrations that are available to the target site within the CNS. Instead, an increase in the total brain concentrations is largely driven by non-specific binding to brain tissue becoming more extensive, typically as a direct result of the higher lipophilicity of the compounds.

Because optimizing the ratio of total brain to total plasma concentrations (Kp) can be very misleading (Jeffrey and Summerfield 2010), an alternative parameter has evolved that is directly related to the unbound brain concentrations and thus a more meaningful parameter to assess the extent of brain penetration (Reichel 2009). This parameter is the ratio of the unbound concentrations in brain to the unbound concentrations in plasma, the so-called Kpuu (Hammarlund-Udenaes et al. 2008). While Kp often is strongly dominated by nonspecific binding to brain tissue constituents, Kpuu is purely dependent on transport process across the BBB, with values greater than 1 suggesting that uptake transport drive the transfer of the drug from blood to brain, while values lower than 1 point to the drug being effluxed out of the brain back into the blood circulation. Kpuu values of around 1 are indicative of passive diffusion dominating the BBB transfer suggesting unrestricted CNS penetration (Reichel 2015).

The rapid and wide acceptance of Kpuu as the parameter of choice to estimate brain penetration has been possible through the existence of in vitro and in vivo methods that are readily available, and a NeuroPK con-

cept that integrates the results into a holistic concept. This allows to link the PK of a compound in plasma to its PK in the CNS, and by incorporating this information into PK/PD relationships further to pharmacodynamic readouts and efficacy endpoints.

In vitro methods include assays related to the rate of permeation, e.g. across the MDCK-MDR1 cell line, and assays related to the extent of brain penetration, e.g. equilibrium dialysis of plasma and brain homogenate or the brain slice technique. The latter not only allows estimation of Kpuu, but also to predict the unbound concentration in brain cells (Loryan et al. 2013), which is important if the target site resides within cells rather than in the brain interstitium.

In vivo methods include the determination of Kp with brain and plasma samples taken at several time points to estimate the area under the concentration-time curve (AUC) in both brain and plasma, the measurement of CSF levels and if feasible brain microdialysis, all of which are performed typically in rodents, ideally the same species as the pharmacology or disease model is based on.

The integrated concept uses the following equations to assess the NeuroPK of a compound:

$$Kp = AUC,brain/AUC,plasma \quad (1)$$

$$Kpuu = Kp * fu,brain/fu,plasma \quad (2)$$

$$Cu,brain = Cu,plasma * Kpuu \quad (3)$$

$$RO = Cu,brain / (Cu,brain + Kd) \quad (4)$$

Equation (3) allows now to estimate unbound brain concentrations from the unbound plasma concentrations using the Kpuu parameter to correcting for the extent of brain penetration across the BBB. In turn, the unbound brain concentrations together with a suitable potency reference (e.g. Kd) can be used to estimate the level of receptor occupancy (RO) which may have been achieved. This estimate of target engagement can be linked to the response level observed in the animal disease model.

Equation (3) also gives rise to approaches to either maximize or minimize unbound brain concentrations. While the former can be achieved by optimizing unbound plasma levels and bringing Kpuu close to 1, i.e. through increasing the rate and extent of oral absorption, decreasing the intrinsic metabolic clearance and removing any recognition of the drug by efflux pumps both at the level of the intestines and at the BBB. In contrast, deliberately designing in some affinity for efflux systems at the BBB that lead to very low Kpuu va-

lues, has been a successful strategy to restrict the action of drugs to the body's periphery and to significantly reduce centrally mediated side effects. A well-known example for this strategy are the second generation antihistamines which do not suffer from the CNS sides effects as opposed to the first generation antihistamines. This mechanistic explanation was first described by David Begley in 2001 (Chishty et al. 2001).

In fully acknowledging the 'free drug hypothesis' (Smith et al. 2010) the integrated NeuroPK framework is consistent with the exposure-centered approach to support drug discovery and development by quantitatively linking potency, target exposure, mode-of-action and efficacy using PK/PD modelling (Reichel and Lienau 2015).

The concept has been shown to be amenable for the hypothesis-driven rational optimization of lead structures in the context of design-make-test-analyze learning cycles in the drug discovery process (Rankovic 2015). Recent work is addressing the translatability from animal to human, and from the healthy situation to disease states (Deo et al. 2013). In addition, differences in the penetration and distribution between different regions within the brain are getting into the focus of the attention (Loryan et al. 2016).

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Joint list of publications to mark the special occasion of the retirement of David J. Begley who together with Prof. Armin Ermisch has been the supervisor of my PhD thesis and ever since remained a friend and a continuous source of inspiration to my work

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PHYSICO-CHEMICAL CONSIDERATIONS FOR SMALL MOLECULE ENTRY TO THE CNS

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The extent and kinetics of plasma-to-brain distribution of a drug are determined by many factors. First, they depend on the plasma-concentration time curve and the kinetics of plasma-protein binding of the drug, together determining the availability of the free drug at the blood-brain barrier (BBB). Here the real challenge starts. Drugs that are bioavailable after oral administration have successfully crossed the intestinal barrier, a process that is for most drugs predictable from physico-chemical properties. Why have so many of these drugs limited access to the brain? Can this also be predicted from their physico-chemical properties?
Some answers result from the comparison of the two barriers. The tight junctions of the intestinal epithelium allow paracellular diffusion of hydrophilic compounds up to a molecular weight of ~300 Da [1]. The endothelial cells of the BBB are so tightly connected that this pathway is negligible.
Kinetics of lipid bilayer permeation should be similar for the two barriers, taking into account the differences in pH and barrier area/fluid volume ratio [2]. Considering that many drugs are weak bases with a pKa > 7 and that the neutral species generally permeates better than the charged one, average permeability should even be higher at the BBB than the intestinal barrier. We have recently introduced a liposomal permeation assay that allows to determine the kinetics of lipid bilayer permeation for weak acids and bases [3]. As expected, permeability increased with lipophilicity. Other parameters such as H-bond donor or acceptor count and molecular weight had a weaker or no effect (unpublished data). This was in particular so when beyond-rule-of-5 compounds were included. Molecular dynamics simulations by Bemporad et al. [4] suggested an interplay between the formation of intramolecular H-bonds and the adaptation of molecular shape to simultaneously reduce the net energy cost of partitioning from water into the hydrophobic environment and the steric constraints by the lipid acyl chains. This could add to the favourable properties of beyond-rule-of-5 compounds regarding intestinal absorption [5].
Efflux transporters at the intestinal barrier and the BBB, in particular P-glycoprotein, are competing with lipid bilayer permeation. A major difference between the two barriers is the drug concentration. Drug concentrations in the intestine can easily saturate efflux transporters, reducing their influence on absorption. Saturating concentrations in the plasma would be in the toxic range for most drugs. Many models exist to predict transport by P-glycoprotein based on physico-chemical and structure-based properties. However, correlations are challenged by the fact that P-glycoprotein transport efficiency depends on the kinetics of competing lipid bilayer permeation. P-glycoprotein substrates are generally lipophilic and have a moderate to high count of H-bond acceptors [6]. Furthermore, P-glycoprotein efflux efficiency increases with increasing molecular weight [7]. Indeed, many beyond-rule-of-5 drugs are good substrates of P-glycoprotein.

Nutrients gain access to the brain by dedicated carrier proteins. The amino-acid like CNS-active drugs L-DOPA, baclofen and gabapentin cross the BBB via the amino acid transport system LAT1. Prodrug approaches aim to target highly expressed carriers at the BBB. In these cases the physico-chemical and structure properties are defined by the targeted carrier.

Considering the high complexity of influencing factors it would come as a surprise if BBB permeation would be easily predictable from the physico-chemical properties of a compound. However, some gross physico-chemical properties can be identified [7-9]. Increasing the number of H-bond donors and acceptors reduces brain uptake. Lipophilicity correlates positively with brain uptake, at least up to a certain logP or logD and polar surface area correlates negatively. A very high fraction of charged species reduces brain uptake. Some parameters increase both passive uptake and P-glycoprotein efflux, among them molecular weight. Many complex in silico models are available but often difficult to interpret (for review, see [9]).

A more promising approach than to predict brain uptake as a whole is to characterise the physico-chemical properties for individual processes and integrate them properly weighted to an overall prediction. Considering the challenge to predict some processes from physico-chemical parameters, the most successful models will probably be those that combine physico-chemical with in-vitro determined parameters, in particular regarding the effects of transport proteins [10, 11].

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UPTAKE AND DISTRIBUTION OF APOE-TARGETED HUMAN SERUM ALBUMIN NANOPARTICLES AND THEIR DELIVERY TO NEURONS IN THE MOUSE BRAIN

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We have recently demonstrated with colleagues at Goethe University, Frankfurt that human serum albumin (HSA) nanoparticles of 200nm average diameter, targeted with recombinant human ApolipoproteinE (ApoE) or ApoA, are transcytosed by the BBB endothelium and delivered to neurons, within 30min [1]. Endocytosis and transcytosis of modified HSA nanoparticles appears to be mediated via LRP1 and 2 receptors, and possibly other receptors, expressed at the luminal surface of the BBB [2]. The major question arises of how these large nanoparticles distribute within the brain. The parenchymal extracellular space is estimated by Thorne and Nicholson (2006) to be 38-64nm in width and these HSA nanoparticles greatly exceed this. Our hypothesis is that the particles are taken up by glial cells, distributed around the brain by cytoplasmic flow in the glial processes and transferred to neurons [3]. Astrocytes are well placed to do this as their end feet ensheath the BBB forming capillaries and their processes ramify throughout the entire brain.

We synthesize HSA nanoparticles using the desolvation technique [4] and modify them with ApoE or Polysorbate80 coating prior to intravenous injection into C57BL/6 mice. Cardiac perfusion fixation is followed by brain processing for electron microscopic and confocal imaging.We showed that ApoE and Polysorbate80 modified HSA nanoparticles are transcytosed by the endothelium of the BBB and delivered to neurons upon intravenous injection. Transcytosis is followed by a rapid distribution through brain tissue and modified 200nm HSA nanoparticles were found over 4µm, measured as a straight line, from the BBB luminal surface, within 30min of injection (Fig. 1). Cytoplasmic transport along astrocytic processes would explain this rapid distribution which cannot be achieved by diffusion and bulk flow in the tortuous parenchymal extracellular space, even by small molecules . We have yet to observe these

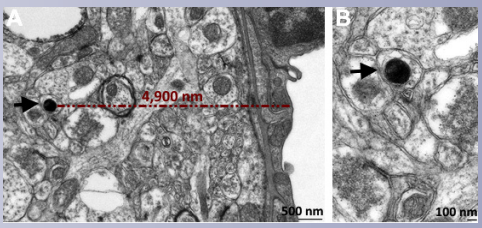

Fig. 1. Polysorbate 80 coated HSA nanoparticle in cortical section 30 min after systemic injection

ApoE-targeted HSA particles within brain parenchymal extracellular space after intravenous administration. 200nm diameter pegylated polystyrene nanoparticles have been shown to have a very limited movement after intraparenchymal CNS injection in mice [5].
These targeting strategies, using BBB apolipoprotein transcytotic mechanisms, may provide a rapid and efficient route for the delivery of a variety of large molecules and drug delivery constructs throughout the brain for therapies which do not normally have a significant brain delivery and distribution.

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THE ROLE OF THE EUROPEAN NEUROLOGICAL SOCIETY IN THE PROMOTION OF RESEARCH AND CARE OF RARE NEUROLOGIC DISEASES

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Rare Neurological diseases are a Pandora Box for Neurology.
The list of the Rare diseases encloses more then 5000 disorders, half of them have a neurological interest, with involvement of the Central and Peripheral nervous system or Muscle or all.
They are underdiagnosticated and a global effort is necessary to improve their knowledge, the possibility to have a correct diagnosis by dissemination of information and culture on them and research, leading to possible treatments (the majority of them are without treatments and in all countries has started a cooperative effort for "orphan drugs".
In USA, since 30 years ago has been stimulated the interest on these disorders, followed 10 year later by the European Community.
Several Scientific Societies have started to have a promoting role on this field.
Since Neurology , as speciality, has the major role in the diagnosis and care of this disease, and basic and applied neurosciences in the research on their pathogenesis, EAN (European Academy of Neurology) have the main responsibility for the promotion of the knowledge of these disorders, of the informations and of the research within the neurological community in Europe.
The Scientific Committee of the EAN have organized a Task force on Rare Neurologic Diseases that will have a strict relationship with the Subspecialities Panels.
The Task Force on Rare Neurological Diseases (WG-NeuRare) will be formed by members from all the different Panels (the Chairmen (ex officio), another member and a delegate from the Patient Associations), open also to Neurologists in Training.
This could be an interesting action of the EAN Board, either from the political and ethical point of vue (orphans diseases and orphan drugs) or from a practical point of vue, giving to our members facilities to be informed on this topics and stimulating interactions for the different groups in Europe involved into research .
The aims of the Task Force will be:

- Stimulation the redaction of a list of Rare Neurological Diseases, with main symptoms and diagnostic criteria and guidelines for diagnosis

- Evaluation of the facilities for diagnosis of Rare Neurologic Diseases (RND) in Europe (a list of facilities and address), with the indication where are the main centers interested in the different disorders, where is possible to do the genetic, biochemical and other laboratory tests, etc
- Promotion of an analysis of the attitude of European Neurologist to RND and which is the state of the art of this issue in the different European Countries;
- Stimulation to promotion of registries for RND, data bank and biobanks. These are main aims of the EU, with Research projects in the Biomed Program.
- Stimulation to create European Networks for RND for diagnosis and research.
- Promotion of Teaching courses in Europe.
- Information Service for Rare Neurological Diseases, within the EAN, that will be able, with the collaboration of the different experts present in the WG, to answer to questions from patients, families and doctors (on line). Information service on new data, new findings, research founds, treatments, etc. Discussion on Rare Cases, within the Section on Web page where cases will be described and experts from SSP will answer.

With this activity, the EAN recognizes the primary role of neurologists in the care of these disorders, the necessity to improve the level of the organization of the Neurological Units in Europe and of the formation of neurologists in the care of rare neurological disorders. But also we will stimulate a better integrated relationship with Patient Associations.

THE RARE METABOLIC DISEASES EUROPEAN REFERENCE NETWORK (METABERN) ACTION

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In light of the existing Commission initiative in the area of rare diseases and to ensure that affected patients are given the priority they deserve and that their needs in receiving better diagnosis and disease prevention, treatment and management are met, we have initiated a series of activities for the establishment of an European Reference Network for METABOLIC DISEASES (MetabERN).
The major goal of this initiative is to ensure a coordinated action in creating the widest collaboration among pediatric and adult metabolic physicians at EU level and facilitating patient access to specialists with expertise in the metabolic field. MetabERN will in fact serve as a referral hub to ensure optimal knowledge-sharing, to improve prevention, early diagnosis and treatment of metabolic diseases at EU level, to bring expertise at patient´s bed, to facilitate access to therapy and to coordinate clinical services to rationalize the existing resources at European level.
The MetabERN is intended to serve all the patients affected by metabolic diseases without distinction of disease.
Today MetabERN involves about 50 major hospitals from 15 different EU countries. Our intention is now to expand the network following the principle of non-exclusion in order to get all the main metabolic pediatric and adult centres, endorsed by local national Authorities, to participate to the MetabERN initiative.

For more information contact the MetabERN coordinator:
Prof Maurizio Scarpa at maur.scarpa@gmail.com

HISTOLOGICAL CHANGES ASSOCIATED WITH DISEASE PROGRESSION IN THE NEUROVASCULAR UNIT OF THE MPSII MOUSE

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Magnetic resonance imaging studies demonstrate gross abnormalities in the perivascular space of Mucopolysaccharidosis type II (MPSII) patients. The goal of the current study was to understand the natural history of any perivascular abnormalities in the MPSII mouse model (IKO). Brains from ~6 week old (mild storage burden) and ~37 week old (high storage burden) IKO mice were collected. Histopathological analysis of the neurovascular unit was completed and compared to wild type animals of the same age. While H&E staining did not reveal obvious morphological changes compared to wild type, there were high levels of LAMP-1 immunoreactivity concentrated around the vascular structure throughout the brains of the IKO mice. This change became more pronounced with age. In addition, the IKO animals showed striking changes in other important cell types of the neurovascular unit, such as astrocytes, and these changes also intensified with disease progression. Taken together, the current data suggest the neurovascular unit becomes increasingly abnormal as part of the disease progression in the IKO mouse model. The current data have important implications in terms of understanding the natural history of the clearance of lysosomal storage through the neurovascular system. Further, these data are also informative in the context of therapeutic approaches for treating MPSII in the central nervous system.

CROSSING THE BARRIER AND MEANING IT: EVALUATION OF A NOVEL SUBSTRATE REDUCTION THERAPY IN GAUCHER DISEASE TYPE 3

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Gaucher disease (GD) is caused by reduced activity of acid β-glucosidase (also known as glucocerebrosidase [GCase]), which results in accumulation of non-metabolized substrates primarily in viscera (non-neuropathic), and less frequently in the CNS (neuronopathic), a traditional classification in this disease with a wide spectrum of severity. Enzyme replacement therapy has been used to successfully treat the visceral manifestations of GD for over 20 years; however currently no direct and effective treatment for CNS disease in Gaucher has been approved. Substrate reduction therapy (SRT) through inhibition of glucosylceramide synthase (GCS) has also been shown to improve the visceral aspects of non-neuronopathic GD, but crossing the blood brain barrier effectively and eliciting measurable response remains an important challenge. Here, we describe the use of a novel inhibitor of GCS with CNS access in mouse models of neuronopathic GD. In the 4L;C* mouse, CNS gliosis and elevated substrate levels (glucosylceramide and glucosylsphingosine) occur prior to death at ~ 45 days. Oral administration of a CNS-accessible GCS inhibitor delayed CNS histopathologic findings and substrate accumulation with a concomitant ~ 40% increase in lifespan. In the conduritol B epoxide (CBE)-induced mouse model of neuronopathic GD, similar gliosis, accumulation of lipids and ataxia were observed. SRT resulted in attenuation of all the neuropathologic manifestations in the continuously CBE treated mouse including astrogliosis, microgliosis, substrate accumulation and ataxia. These results strongly support the development of SRT for the treatment of neuronopathic GD.

PRECLINICAL RESULTS OF HEPARAN SULFATE CONTENT IN THE BRAIN FOLLOWING INTRAVENOUS SBC-103 ADMINISTRATION IN A MUCOPOLYSACCHARIDOSIS IIIB MOUSE MODEL AND INITIAL, 24 WEEK RESULTS OF HEPARAN SULFATE LEVELS IN CEREBROSPINAL FLUID (CSF) AND SERUM IN AN OPEN LABEL, PHASE I/II, FIRST-IN-HUMAN CLINICAL TRIAL OF INTRAVENOUS SBC-103 IN MUCOPOLYSACCHARIDOSIS IIIB PATIENTS

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Mucopolysaccharidosis IIIB (MPS IIIB; Sanfilippo syndrome type B) is caused by a marked decrease in alpha-N-acetyl-glucosaminidase (NAGLU) enzyme activity which leads to the accumulation of heparan sulfate (HS) in the brain and other organs, progressive brain atrophy, neurocognitive decline and behavioral disturbances. At present there is no treatment for this disorder. We report preclinical results of HS levels and NAGLU enzyme activity in brain tissue in a MPS IIIB mouse model [NAGLU(-/-)] and initial results from the ongoing first-in-human (FIH) trial (NCT02324049).

In a preclinical study in the MPS IIIB mouse model [NAGLU(-/-)], SBC-103 [5, 10, and 20 mg/kg body weight (4-6 doses)] was administered intravenously (IV) once weekly. Significant reduction of HS in the brain was seen at all doses. Total HS percent change from baseline (mean [SD]) was -60 [21], -60 [12], and -75 [9] for 5, 10, and 20 mg/kg groups, respectively. These HS decreases in the brain were accompanied by demonstrable increases (7-10 folds) in brain SBC-103 (NAGLU) enzyme activity.

In the FIH trial in MPS IIIB patients, SBC-103 (0.3, 1, and 3 mg/kg) was administered as an IV infusion every other week in patients aged 2-12 years (developmental age ≥1 year). The primary objective of the trial was assessment of safety and tolerability of IV SBC-103 and secondary objectives included evaluation of effect of SBC-103 on total HS levels in CSF and serum, and pharmacokinetic

(PK) profile of SBC-103. Eleven patients were enrolled (median age 4 years; range 2-10 years) and received IV SBC-103. All 11 patients continue on extended therapy. During the 24 weeks, there were 3 treatment-emergent serious adverse events (SAEs) in a single patient and 6 infusion-associated reactions in 3 patients. No SAEs were considered related to SBC-103. At week 24, total HS percent change from baseline (mean [SD]) in the CSF was 10.9 [14.7], -0.43 [11.9] and -26.2 [20.9] for 0.3, 1, and 3 mg/kg groups, respectively. Total HS percent change from baseline (mean [SD]) in the serum was -39.6 [15.4], -53.9 [19.7] and -40.5 [23.9] for 0.3, 1, and 3 mg/kg groups, respectively. HS reduction in CSF was linearly correlated with SBC-103 serum PK exposures (maximum concentration [Cmax] and area under the curve [AUC]). Serum PK exposures increased linearly with increased doses.

Following IV administration of SBC-103 in a MPS IIIB mouse model, significant reductions in HS in the brain were seen and these were accompanied by demonstrable increases in brain SBC-103 enzyme activity. Initial observations in the FIH trial in MPS IIIB patients suggest that IV SBC-103 was biologically active and well tolerated. Potential blood brain barrier penetration of IV SBC-103 was illustrated by HS reduction in CSF in MPS IIIB patients. These data support further investigation of IV dosing of SBC-103 as a new approach for patients with MPS IIIB.

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ADMINISTRATION OF FUSION PROTEINS INCORPORATING MTFPEP OR MTF IN A LYSOSOMAL ENZYME (I2S) DELIVERS A THERAPEUTICAL CONCENTRATION OF I2S TO THE CNS TO TREAT MPS II (HUNTER SYNDROME)

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biOasis Technologies Inc. is a biopharmaceutical company focused on the treatment of CNS diseases and disorders. The Company is developing proprietary peptide vectors based on melanotransferrin (MTf) for the delivery of therapeutics to the CNS, this platform is called "Transcend".

Drug delivery into the CNS remains a significant challenge for clinical neuroscientists as most drugs show limited penetration in the CNS due to the blood-brain barrier (BBB). The BBB characteristics provide a natural defense against toxic or infective agents circulating in the blood. Therefore, the development of new technologies to cross the BBB for brain parenchyma uptake is of great interest and vital importance for the treatments of neurological disorders and genetic diseases. A family of vectors called Transcend, comprising the full-length MTf and peptides thereof, are developed by biOasis Technologies Inc. to facilitate receptor-mediated drug delivery into the brain to treat CNS disorders.

We have previously shown that lysosomal enzymes, as iduronate 2-sulfatase (I2S), labeled with fluorescent dyes are transported across the BBB in the lysosome of brain cells when incorporating MTf or MTfpep (12 amino-acid peptide) in a fusion protein.

Studies addressing the brain delivery of I2S for the treatment of Hunter Syndrome in k/o mice have shown efficacy in reducing significantly the amount of lysosomal storage in brain cells. Two fusion proteins, composed of I2S incorporating MTf or MTfpep, were administered in k/o mice. High induced I2S activity first shown in an in-vitro assay, was confirmed by the decrease of total GAG content in liver and urine, to wild-type mice level. These data were further confirmed by the analysis of heparan sulfate level in liver, performed by MS technology. Analysis and quantification of organel-

les in brain cells have shown significant decrease of the amount of vacuoles and lysosomes demonstrating a therapeutic concentration of lysosomal enzymes reaching the brain parenchyma. These data will be further discussed in the presentation.
Results show additional application of our platform technology and demonstrate that MTfpep can be used as a carrier capable of shuttling a variety of compounds, ranging from small anti-cancer agents to larger biologics, across the BBB into the brain parenchyma in therapeutic doses, enabling treatment of neurological disorders.

NOTES

THIS WORKSHOP SUPPORTS RESEARCH

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