



Ninth  
European  
Workshop  
and  
InNerMeD  
Information  
Network

Second Open Conference



Frankfurt, Germany,  
Mercure Hotel Frankfurt Airport

**February 5<sup>th</sup> - 7<sup>th</sup>, 2015**

BRAINS  
FOR  
BRAIN



**European Task Force on Brain and  
Neurodegenerative Lysosomal Storage Diseases**

For details see [BRAINS.TCFEBRAIN-Online](http://BRAINS.TCFEBRAIN-Online) / Via Discussion: [TCFEBRAIN@listserv.elsevier.com](mailto:TCFEBRAIN@listserv.elsevier.com) / [www.brainsforbrain.eu](http://www.brainsforbrain.eu) / [info@brainsforbrain.eu](mailto:info@brainsforbrain.eu)

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.

## 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 panel 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 therapeutic 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.

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

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 paediatric neurometabolic diseases.

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, strengthening 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 Copenhagen, 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.

## AIMS OF THE WORKSHOP

The aims of the ninth 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

### Scientific Officer

**Cinzia Maria Bellettato** (IT)

### Logistics

**Jazz Travel & Congress**, Spoleto, Italy

giacomo@jazzitaly.com, p.caprelli@jazzitaly.com

## SCIENTIFIC PROGRAMME 2015

### February 5<sup>th</sup> 2015

**10.30-10.45**

#### WELCOME AND OPENING

**10.45-11.05**

#### OPENING LECTURE

**MARIA DA GRAÇA CARVALHO,**

*Member of the European Parliament*

The effort of the EU Commission for research: the Horizon 2020 programme and the vision for the future  
Discussion.

**11.15-12.00**

#### PLENARY LECTURE

**STEFAN LIEBNER,** *Goethe University,*

*Frankfurt, DE*

Molecular Regulation of Endothelial Barrier Properties in the Central Nervous System  
Discussion.

**12.00-16.15**

#### BASIC ASPECTS

Chair Discussants:

**GERT FRICKER, DE - ROMEO CECHELLI, FR**

**12.00-12.25**

**PAUL SAFTIG,** *Christian-Albrechts-Universität zu Kiel, DE*

Emerging roles of lysosomal membrane proteins in health and disease  
Discussion.

**12.30 LUNCH**

**14.30-14.55**

**JEFFREY ILIFF,** *Oregon Health & Science University, Portland, USA*

Paravascular Cerebrospinal Fluid Recirculation: From Housekeeping to Neurodegeneration  
Discussion.

**15.05-15.30**

**MICHAEL W SALTER,** *Hospital for Sick Children and University of Toronto, CA*

Expanding roles of microglia in chronic pain: implications for therapy  
Discussion.

**15.40-16.05**

**JEAN GRUENBERG,** *University of Geneva, CH*  
Endosomal lipids in trafficking and signaling  
Discussion.

**16.15 Coffee**

**16.30-18.50**

#### BASIC ASPECTS 2

Chair Discussants:

**FRAN PLATT, UK - INGOLF BLASIG, DE**

**16.30-16.55**

**ROMEO CECHELLI,** *University of Artois, FR*  
Cancer Cell Metastasis to the Brain: Necessary Requirement of a Human In Vitro Model Presenting a Blood-Brain Barrier Phenotype  
Discussion.

**17.05-17.30**

**SVITLANA GARBUZOVA-DAVIS,** *University of South Florida, Morsani College of Medicine, Tampa, USA*  
Human Umbilical Cord Blood Cells in Treatment of MPS IIIB  
Discussion.

**17.40-18.05**

**MARIA FRANCISCA COUTINHO,** *Research and Development Unit, Department of Human Genetics, INSA, Porto, PT*  
The role of the mannose-6-phosphate receptor in lysosomal function and dysfunction  
Discussion.

**18.15-18.40**

**MIKA RUONALA,** *NeuroToponomics Group, CMP, University of Frankfurt, DE*  
The quest towards CNL3 function  
Discussion.

**19.30 DINNER**

February 6<sup>th</sup> 2015

**9.00-13.00**  
**BASIC ASPECTS 3**

Chair Discussants:  
**TIMOTHY COX, UK - GREG PASTORES, EIRE**

**9.00-9.25**  
**DANICA B STANIMIROVIC**, *National Research Council of Canada, Human Health Therapeutics Portfolio, Ottawa, CA*  
Mechanisms of antibody trafficking and transcytosis across the blood-brain barrier: A role for extracellular microvesicles?  
Discussion.

**9.35-10.00**  
**RODNEY PEARLMAN**, *President of The Bluefield Project to Cure Frontotemporal Dementia, USA*  
Progranulin Expression and Frontotemporal Dementia (FTD), a link with Neuronal Ceroid Lipofuscinosis (NCL)  
Discussion.

**10.10-10.35**  
**GERT FRICKER**, *Ruprecht-Karls-Universität Heidelberg, DE*  
Drug targeting to the brain by colloidal carrier systems  
Discussion.

**10.45 Coffee**

**11.15-11.40**  
**JÖRG KREUTER**, *Goethe-Universität Frankfurt, DE*  
Drug Delivery to the CNS by Polymeric Nanoparticles: What Do We Know  
Discussion.

**11.50-12.15**  
**ULRICH MATZNER**, *Rheinische Friedrich-Wilhelms Universität, Bonn, DE*  
Anti-inflammatory therapy with simvastatin improves CNS pathology and function in a mouse model of metachromatic leukodystrophy  
Discussion.

**12.25-12.50**  
**TIM SPECTOR**, *Kings College, UK*  
How May Two Individuals, With The Same Genetic Mutation, Display Quite Different Phenotypes  
Discussion.

**13.00 LUNCH**

**14.30-16.15**  
**CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTIC OPTIONS**  
Chair Discussants:  
**TONY FUTERMANN, IL - GENEROSO ANDRIA, IT**

**14.30-14.55**  
**BEVERLY DAVIDSON**, *University of Pennsylvania, Philadelphia, USA*  
Bypassing the BBB: Gene Based Therapies for the Lysosomal Storage Diseases  
Discussion.

**15.05-15.30**  
**ALFRIED KOHLSCHÜTTER**, *University Medical Center Hamburg-Eppendorf, Hamburg, DE*  
Experience with intracerebroventricular delivery of a lysosomal enzyme in the clinical trial for CLN2 disease  
Discussion.

**15.40-16.05**  
**JOSEPH MUENZER**, *University of North Carolina at Chapel Hill, USA*  
Investigational Intrathecal (IT) Enzyme Replacement Therapy for the Severe Form of Hunter Syndrome  
Discussion.

**16,15 Coffee**

**16.45-19.30**  
**CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTIC OPTIONS 2**  
Chair Discussants:  
**ALFRIED KOLSCHUTTER, DE - DANICA STANIMIROVIC, CA**

**16.45-17.10**  
**EMYR-LLOYDS EVANS**, *University of Oxford, UK*  
A Zebrafish model for NPC for phenotyping and drug screening  
Discussion.

**17.20-17.45**  
**JAMES CALLAWAY**, *ArmaGen Technologies, Inc, USA*  
Transition of Antibody Directed Receptor Mediated Transcytosis Therapies into Clinical Studies  
Discussion.

**18.00-18.45**  
**PLENARY LECTURE**  
**ALESSANDRA D'AZZO**, *St. Jude Children's Research Hospital, Memphis Tennessee, USA*  
Lysosomal multienzyme complex: pros and cons of working together  
Discussion.

**20.30 DINNER**

February 7<sup>th</sup> 2015

**08.30-10.00**  
**BRAINS FOR BRAIN AND INNERMED EUROPEAN ACTIONS**  
Chair Discussants:  
**DAVID BEGLEY, UK - MAURIZIO SCARPA, DE**

**8.30-8.45**  
**MAURIZIO SCARPA AND DAVID BEGLEY**, *Brains for Brain Foundation B4B, (IT)*  
Update on the activity of B4B at EU  
Discussion.

**9.00-9.20**  
**MAURIZIO SCARPA** (*Brains for Brain Foundation B4B, IT*) **AND MARIANGELA LUPO** (*Gianni Benzi Pharmacological Research Foundation FGB, IT*) *on behalf of All InNerMeD Project Partners*  
Presentation of the Inherited NeuroMetabolic Diseases Information Network (InNerMeD-I Network) and website platform to increase awareness on Neurometabolic Diseases  
Discussion.

**9.30-9.40**  
**GIOVANNI SMANIA**, *Consorzio per Valutazioni Biologiche e Farmacologiche – CVBF, IT*  
Modelling and simulation, extrapolation

and alternative study designs as innovative approaches to develop paediatric drugs for iNMDs  
Discussion.

**9.45-9.55**  
**VIVIANA GIANNUZZI**, *Gianni Benzi Pharmacological Research Foundation - FGB, IT*  
Impact of the new European Clinical Trial Regulation on iNMDs paediatric research  
Discussion.

**Coffee**

**10.30-13.00**  
**B4B AND BIOTECH COLLABORATIONS**  
Chair Discussants:  
**JOSEPH. MUENZER, USA - DAVID BEGLEY, UK**

**10.30-10.55**  
**ANNE CHRISTIANSEN**, *Drug Discovery and Translational Research, Shire, USA*  
Innovation at Shire – Drug discovery approach for central nervous system targeting  
Discussion.

**11.05-11.30**  
**RICHARD W. D. WELFORD**, *Actelion Pharmaceuticals Ltd, Allschwil, CH*  
Plasma lysosphingolipids as biomarkers for Niemann-Pick disease type C: use in diagnose and potential further applications  
Discussion.

**11.40-12.05**  
**REINHARD GABATHULER**, *biOasis Technologies Inc, CA*  
Using a Peptide derived from Transcend (MTf, p97) to Deliver Biologics to the CNS using a Physiologic Pathway  
Discussion.

**12.15-12.40**  
**MIRKO ESSING**, *BioMarin Europe International, UK*  
Biomarin- Products and Compounds  
Discussion.

**LUNCH AND FAREWELL to the next 2016 meeting (10<sup>th</sup>)**



down of the candidate genes, along with Wnt/ $\beta$ -catenin activation, significantly improved TEER in primary brain endothelial cells as a measure for barrier tightness. These data suggest that Wnt/ $\beta$ -catenin in combination with other pathways regulates barrier function at the aging and the dementia-affected BBB. In summary, we can show that activation of the Wnt pathway in ECs is not only important during early brain vascularization, but also during later stages of life and particularly in the aged CNS. Herein AC-derived Wnts may contribute to brain EC differentiation and vascular remodeling. At the same time the lack of Wnt activation from certain vessels such as those of the CVO is crucial for the formation of their specific phenotype and function.

Further investigations are required to better understand vascular heterogeneity in the brain in general and during aging in particular.

## EMERGING ROLES OF LYSOSOMAL MEMBRANE PROTEINS IN HEALTH AND DISEASE

### PAUL SAFTIG

*Biochemical Institute, Christian Albrechts-Universität Kiel, Olshausenstr. 40, D-24098 Kiel, Germany.*

The lysosomal membrane was thought for a long time to primarily act as a physical barrier separating the luminal acidic milieu of lysosomes and lysosome-related organelles from the cytoplasmic environment. Meanwhile, it has been realized that unique lysosomal membranes play essential roles in a number of cellular events ranging from phagocytosis, autophagy, cell death, virus infection to membrane repair. An overview about the most interesting emerging functions of lysosomal membrane proteins (LMPs) and how they contribute to health and disease will be provided. Their role in acidification, transport of metabolites and ions across the membrane, intracellular transport of hydrolases, lipid transport and the regulation of membrane fusion events has been documented. Studies in patient cells, non-mammalian model organisms and knockout mice contributed to our understanding of how the different lysosomal membrane proteins affect cellular homeostasis, developmental processes as well as tissue functions. More than 150 integral LMPs have been identified but only for a minority of these proteins studies about their biochemistry and function has been reported. New experimental tools, such as electrophysiological recording of lysosomal currents and metabolite transport assays, new animal models as well as genetic studies are useful to fill the gap of knowledge about these fascinating and highly specialized proteins. Although a considerable gain of knowledge about the function of LMPs within the lysosomal membrane has attracted the attention of a wide field of cell biological researchers, we are only at the beginning to fully realize the complexity and molecular players and details of the events regulated by membrane proteins in the interface between the cytosolic and the lysosomal world.

## PARAVASCULAR CEREBROSPINAL FLUID (CSF) RECIRCULATION: FROM HOUSEKEEPING TO NEURODEGENERATION

**JEFFREY ILIFF, PHD<sup>1</sup> AND MAIKEN NEDERGAARD, MD, PHD<sup>2</sup>**

<sup>1</sup> *Department of Anesthesiology and Perioperative Medicine, Knight Cardiovascular Institute, Oregon Health & Science University, Portland OR USA.*

<sup>2</sup> *Center for Translational Neuromedicine, University of Rochester Medical Center, Rochester NY USA.*

Aging is the strongest risk factor for the development of virtually every neurodegenerative disease, including Alzheimer's disease which is characterized histopathologically by the aberrant buildup of mis-aggregated amyloid  $\beta$  and hyper-phosphorylated tau into senile plaques and neurofibrillary tangles, respectively. Yet the changes that occur in the aging brain that render it vulnerable to protein aggregation and neurodegeneration remain unclear.

In a series of recent studies, our group has described a network of paravascular channels that facilitates the recirculation of cerebrospinal fluid (CSF) through the brain, allowing CSF to exchange with the brain interstitial fluid (ISF). Operating chiefly during sleep, this paravascular CSF-ISF exchange facilitates the efficient clearance of interstitial solutes including amyloid  $\beta$  and tau from the brain. Fluid movement along these paravascular pathways is dependent upon the astroglial water channel aquaporin-4 (AQP4), which is expressed along perivascular astrocytic endfeet that ensheath the cerebral vasculature.

In the aging mouse brain, we observe that paravascular CSF-ISF exchange is dramatically impaired as is the clearance of interstitial amyloid  $\beta$ . We observe that the perivascular localization of AQP4, which is a characteristic feature of this water channel, is lost in the aging brain. Using a transgenic mouse line that lacks perivascular AQP4 polarization, we observe that even in the young brain, mis-localization of AQP4 is sufficient to impair paravascular CSF-ISF exchange. Consistent with these findings in mice, we observe in human brain tissue that AQP4 localization is altered in the aging frontal cortex and that the loss perivascular AQP4 is associated with increasing amyloid  $\beta$  plaque burden and Braak staging of neurofibrillary tangle pathology. These findings suggest that the perivascular localization of AQP4 organizes efficient fluid flow through the brain along the vasculature, and that the loss of this organization may be one of the factors that renders the aging brain vulnerable to protein aggregation and neurodegeneration.

These findings have three important implications. First, they suggest that maintaining paravascular CSF-ISF exchange, perhaps through targeting perivascular AQP4 localization, may offer a novel therapeutic approach to the prevention or treatment of neurodegenerative diseases such as Alzheimer's disease. Second, it suggests that the failure of paravascular CSF-ISF exchange in the aging or the injured brain may be an early contributing event in the development of Alzheimer's disease or other neurodegenerative conditions. Thus defining biomarkers, including imaging biomarkers, to detect impairment of these processes may allow us to evaluate the vulnerability of the brain to protein aggregation, perhaps years before the onset of amyloid  $\beta$  deposition. Lastly, because these paravascular pathways constitute routes for the distribution of drugs arriving in the CNS across the blood brain barrier, through convection-enhanced delivery, or through the CSF, understanding the features of these pathways and how they change in pathology may substantially improve our ability to effectively deliver therapeutic molecules into brain tissue for the treatment of a wide variety of neurological disorders.

## EXPANDING ROLES OF MICROGLIA IN CHRONIC PAIN: IMPLICATIONS FOR THERAPY

**MICHAEL W. SALTER MD PHD**

*Hospital for Sick Children and University of Toronto - Toronto, Canada*

Neuron-microglial interactions are increasingly recognized as being key for physiological and pathological processes in the central nervous system. Microglia have been found to play a causal role in neuropathic pain behaviours resulting from peripheral nerve injury, and a core neuron-microglia-neuron signaling pathway has been elucidated. Within the dorsal horn of the spinal cord, microglia suppress neuronal inhibition by a cascade involving activation of microglial P2X4 receptors causing the release of brain derived neurotrophic factor (BDNF). BDNF acts on trkB receptors which leads to a rise in intracellular chloride concentration in dorsal horn nociceptive output neurons. In addition to suppressing inhibition, peripheral nerve injury causes activity-dependent facilitation at dorsal horn glutamatergic synapses which enhances nociceptive transmission. This enhancement is mediated by intracellular signaling networks involving serine/threonine and tyrosine kinases within nociceptive transmission neurons. Key for this enhancement is facilitation of NMDA receptor function by Src family tyrosine kinases. The suppression of inhibition and facilitation of excitation in transforms the activity and response characteristics of nociceptive transmission neurons in the spinal cord. Recently we have discovered that microglia-to-neuron signaling is not only critical for pain hypersensitivity after peripheral nerve injury but also for the paradoxical hyperalgesic effect of morphine and other opioids. We anticipate that by targeting microglia-neuron signaling pathways new therapeutic strategies for chronic pain as well as its comorbid sequelae may be developed.

*Funding: Supported by CIHR, Krembil Foundation, Canada Research Chairs and Anne and Max Tanenbaum Chairs.*

## ENDOSOMAL LIPIDS IN TRAFFICKING AND SIGNALING

**CAMERON SCOTT\*, DIMITRI MOREAU\*\* , STEFANIA VOSSIO\* , FABRIZIO VACCA\* ,  
MARC CHAMBON‡, GERARDO TURCATTI‡ AND JEAN GRUENBERG\***

*\* Department of Biochemistry, University of Geneva, Switzerland*

*‡ Biomolecular screening facility-ACCESS, EPFL & University of Geneva, Switzerland.*

Most mammalian cells acquire cholesterol from extracellular sources, via receptor-mediated endocytosis of low-density lipoproteins (LDL). After internalization, the low early endosomal pH uncouples the LDL particles from their receptor. LDLs are then packaged into endosomal transport intermediates and eventually delivered to late endosomes. In this compartment, after de-esterification of LDL cholesteryl esters, free cholesterol is released and exported from endosomes to other cellular destinations by mechanisms that remain ill defined. It is clear however that disruption of this process has profound consequences for cellular sterol homeostasis and can result in debilitating human pathologies such as the neurodegenerative disease Niemen-Pick C (NPC). Our previous studies suggest that the fate of LDL-derived cholesterol is linked to the unconventional phospholipid lysobisphosphatidic acid (LBPA) that is abundant in late endosome intraluminal membranes, since interfering with LBPA functions phenocopy NPC at the cellular level. Data from others and us also suggest that some pathogens, including vesicular stomatitis virus (VSV), gain entry into the host cell cytoplasm by hijacking the same pathway as used by cholesterol during export. To better understand the mechanisms of endosomal cholesterol transport, we initiated RNAi and compound high content imaging-based screens to identify gene products that control lipid distribution and amounts. These unbiased screens implicate the Wnt signaling pathway as a potent regulator of cholesterol homeostasis, and suggest that LBPA itself is under the direct control of a poorly characterized signaling pathway. The possible roles of these pathways in lipid regulation will be discussed.

**CANCER CELL METASTASIS TO THE BRAIN:  
NECESSARY REQUIREMENT OF A HUMAN IN VITRO MODEL  
PRESENTING A BLOOD-BRAIN BARRIER PHENOTYPE**

**ROMEO CECHELLI<sup>1</sup>, AURORE DROLEZ<sup>1</sup>, ELODIE VANDENHAUTE<sup>1</sup>, MARIE-PIERRE DEHOUC<sup>1</sup>, SYLVAIN JULIEN<sup>2</sup>, PHILIPPE DELANNOY<sup>2</sup>, CAROLINE MYSIOREK<sup>1</sup>**

<sup>1</sup> UArtois, LBHE, EA 2465, F-62300, Lens, France.

<sup>2</sup> Unité de Glycobiologie Structurale et Fonctionnelle, UMR CNRS 8576, F-59000, Villeneuve d'Ascq, France.

Among breast metastatic cancer patients, around 7-17% will develop brain metastases, associated with a poor prognosis due to the survival rate at one year lower than 20%. To reach the brain parenchyma, cancer cells need to cross the highly restrictive endothelium of the BBB. Preventing cancer cells from reaching the brain could provide a relevant strategy, due to the low efficiency of brain metastasis treatments. To achieve this, identifying the mechanisms of interaction that occur between cancer cells and BBB endothelium is an essential requirement. To study and understand these mechanisms, an in vitro approach seems to be ideal; however, the use of a model that fulfills all the BBB characteristics - including tight junctions and low permeability - is indispensable. Therefore, we used several in vitro models from various origins (bovine, mouse, human) in order to find the most appropriate tool to study interactions between breast cancer cells and BBB endothelial cells.

We focused on the adhesion of breast cancer cells to the endothelium. Two breast cancer cell lines were used: the MDA-MB-231 cell line corresponding to an aggressive type of cancer that easily metastasizes to the brain and the weakly metastatic MCF-7 cell line. We compared the adhesion of these 2 cell lines on different in vitro models, corresponding to endothelial cells from various origins cultivated alone or with pericytes or glial cells, known to induce BBB characteristics, and for which we previously evaluated BBB properties.

According to our results, only our human in vitro BBB model (Cecchelli et al 2014), based on the coculture of endothelial cells generated from cord blood stem cells and brain pericytes, provided both adhesion differential representative of the cancer cell lines relative aggressiveness and BBB properties amount to in vivo data. So, this model will be the most suitable to study interactions between breast cancer cells and BBB endothelial cells.

1 - Cecchelli R., Aday S., Sevin E., Almeida C., Culot M., Dehouck L., Coisne C., Engelhardt B., Dehouck M.-P. and Ferreira L. *A stable and reproducible human blood-brain barrier model derived from hematopoietic stem cells*. PlosOne 2014 June 9-6

**HUMAN UMBILICAL CORD BLOOD CELLS IN TREATMENT OF MPS III B**

**SVITLANA GARBUZOVA-DAVIS, ALISON E. WILLING AND PAUL R. SANBERG**

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Sanfilippo syndrome type B (MPS III B) is an inherited disorder caused by a deficiency of the alpha-N-acetylglucosaminidase (Naglu) enzyme in the degradative pathway of heparan sulfate (HS). There are no treatments available for this disease. Our previous studies showed that a single administration of mononuclear human umbilical cord blood (MNC hUCB) cells into the cerebral ventricle of pre-symptomatic Naglu mice or intravenous cell delivery at different stages (early symptomatic or late stage) of disease had a beneficial effect, probably due to enzyme delivery into the enzyme-deficient mutant mice. Transplanted mutant mice showed cognitive improvement and decreased disease-related hyperactivity even when cells were administered in late stage of disease. After intracerebroventricular or intravenous administrations of MNC hUCB cells, the cells were found widely distributed within and outside the CNS. HS accumulation was significantly reduced in the liver and spleen of Naglu treated-mice. However, most observed behavioral benefits in Naglu mice were limited to the first months after cell transplantation, possibly due to declining production of the missing enzyme over time. In consideration of this point, our pre-clinical translational study was designed to determine the effect of repeated doses of intravenously administered MNC hUCB cells into early symptomatic Naglu mice during a period of 6 months. Results showed significant behavioral improvements in mutant mice with repeated cell transplants. The repeated cell administrations restored hippocampal cytoarchitecture, protected the dendritic tree, decreased GM3 ganglioside accumulation and decreased microglial activation, particularly in hippocampus and cortex. Immunohistochemical analysis showed more migrating cells in the brains of multiple-cell treated mutant mice than in animals receiving a single cell injection. The majority of cells were found in the cerebellum, medulla, cerebral cortex, thalamus, and olfactory bulb of multiple-cell treated mutant mice and some cells expressed nestin. Administered cells were also widely distributed in the abdominal organs and peripheral blood. Another advantage of repeated cell injections was significantly reduced HS accumulation in livers of Naglu mice versus single-cell-treated or non-treated mutants. These results suggest that the neuroprotective effect of transplanted cells can be enhanced by repeated administrations. Thus, repeated intravenous administrations of MNC hUCB cells into Naglu mice have a

prolonged beneficial effect compared to a single administration, most likely due to continuous enzyme delivery into the enzyme-deficient mutant mice. Overall, results of our pre-clinical translational studies demonstrate the potential of hUCB cells in the treatment of Sanfilippo Syndrome type B. This treatment might be also suitable for genetic disorders such as glutaric acidemia, Friedreich's ataxia, and others.

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## THE ROLE OF THE MANNOSE-6-PHOSPHATE RECOGNITION MARKER IN LYSOSOMAL FUNCTION AND DYSFUNCTION

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To accomplish their degradative function lysosomes must be filled with specific proteins, which after being synthesized in the endoplasmic reticulum (ER) have to be directed to the trans-Golgi network (TGN) for further processing and lysosomal targeting. The explanation of how lysosomal enzymes are accurately recognized and selected over many other proteins in the TGN relies on a small recognition marker, added exclusively to their N-linked oligosaccharides as they pass through the cis-Golgi: the mannose 6-phosphate (M6P) group.

Generation of the M6P recognition marker depends on a two-step reaction involving two different enzymes: UDP-N-acetylglucosamine 1-phosphotransferase (GlcNAc-phosphotransferase) and  $\alpha$ -N-acetylglucosamine-1-phosphodiesterase  $\alpha$ -N-acetylglucosaminidase (uncovering enzyme). GlcNAc-phosphotransferase catalyses the transfer of a GlcNAc-1-phosphate residue from UDP-GlcNAc to C6 positions of selected mannoses in high-mannose type oligosaccharides of the hydrolases. Then, the uncovering enzyme removes the terminal GlcNAc, exposing the M6P recognition signal. At the TGN, the recognition signal allows the segregation of lysosomal hydrolases from all other types of proteins through selective binding to the M6P receptors: the cation-independent M6P receptor (CI-MPR) and/or the cation-dependent M6P receptor (CD-MPR). The produced clathrin-coated vesicles bud off from the TGN and fuse with late endosomes (LE). At the low pH of the LE, the hydrolases dissociate from the M6P receptors and the empty receptors are recycled to the Golgi for further rounds of transport.

Impairments in this delivery/transport pathway may result in missorting of lysosomal enzymes, with consequent severe pathological condition. Additionally, the expression levels of M6P pathway functional components and of the recognition marker itself may have an influence on the efficacy of some therapeutic approaches.

Here we will review the current knowledge on each of the major proteins involved in the M6P-dependent pathway, highlighting their involvement in disease. Special attention will be given to the lysosomal storage disorders associated to GlcNAc-phosphotransferase loss of function: Mucopolipidosis type II and III.

## THE QUEST TOWARDS CLN3 FUNCTION

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20 years after acknowledging the mutations in the *Cln3* gene responsible for the Juvenile Neuronal Ceroid Lipofuscinosis (JNCL; NCL3; Batten disease) the structure, cellular localization or function of the full-length CLN3 protein are still unclear. This is the major hurdle en route to understand the JNCL disease mechanisms and development of therapeutic options, and partly arises from the lack of cell biology tools, such as antibodies and functional, genetically modified CLN3 forms. As part of a larger project we addressed this general problem with two unconventional approaches. First, using a cloning strategy based on Tn5 transposase enzyme we generated a library of hCLN3 clones with a randomly inserted eGFP or a myc-tag. The different clone variants were tested for functionality by assessing their ability to restore a known lysosomal phenotype in cerebellar granule neurons derived from a homozygous *Cln3Dex7/8* mouse model. The clones with significant rescue potential were used in structural analyses that suggested a novel CLN3 membrane topology model. The clones are also being used in studies aiming to reveal the functional modus of CLN3. We then utilized the acquired structural knowledge and screened the phage display of llama single-domain antibody fragments (Variable domain of the heavy chain; VHs) for human CLN3 binders. From the 1012 different single-chain VHH variants a dozen of VHH variants binding either to the amino acid sequence 1-33 or 59-101 of human CLN3 were identified, sequenced, and expressed in *E. Coli* as myc-, his-, and biotinylated variants. Preliminary tests imply anti-hCLN3 VHH's as potentially useful tools for standard proteomic studies, and in immunofluorescence analyses.

## MECHANISMS OF ANTIBODY TRAFFICKING AND TRANSCYTOSIS ACROSS THE BLOOD-BRAIN BARRIER: A ROLE FOR EXTRACELLULAR MICROVESICLES?

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Delivery of therapeutic antibodies into brain parenchyma can be achieved by the receptor-mediated transcytosis (RMT) across the blood-brain barrier (BBB). Mechanisms of RMT are still poorly understood and may depend on the RMT receptor engaged by the antibody. Current methods for examining endosomal antibody sorting are either non-quantitative or require antibody labeling with tracers. We have developed a multiplexed quantitative method to interrogate and quantify antibody sorting and trafficking through various intracellular and extracellular brain endothelial cell (BEC) compartments. The method involves endosome fractionation and label-free targeted mass spectrometry to quantify both the antibody and its RMT receptor. To facilitate BBB-crossing antibody engineering strategies, the method was used to evaluate the role of antibody valency and the presence of Fc fragment in directing intracellular sorting of antibodies engineered with a BBB-crossing single-domain antibody (sdAb) FC5. Rat BEC were co-incubated with FC5 variants (monomeric FC5, mono-valent FC5Fc or bi-valent FC5Fc) and/or control antibodies (non-internalizing A20.1 sdAb, internalizing anti-EGFR sdAb EG2, and rat transferrin receptor antibody OX-26) for 45 min. Endosomes were isolated and separated using sucrose-density gradient and ultracentrifugation. Twenty fractions were collected, processed and analyzed using multiplexed nanoLC-MRM to simultaneously quantify specific endosomal markers, RMT receptors and co-incubated antibodies in each fraction. Extracellular microvesicles were also isolated and analysed by proteomic methods.

Multiplexed MRM quantitation showed early endosome marker enrichment in high-density (HDF) and late endosome/lysosome markers in low-density (LDF) fractions. Examined antibodies showed the following distribution ratios between HDF (early endosomes) and LDF (late endosomes/lysosomes), respectively: A20.1 – no internalization; EG2 - 33:66; OX-26 (IgG) -63:37; monomeric FC5 (VHH) - 65:35; monovalent FC5Fc -84:16; bi-valent FC5Fc - 91:8.

The ranking of apparent permeability coefficients (Papp) in in vitro rat BBB model (A201=EG2 (both very low) <OX26<FC5<monoFC5Fc<bi-FC5Fc) indicated a strong direct correlation between antibody sorting into early endosomes and its transmigration across BEC monolayer. BEC extracellular microvesicles were shown to contain several RMT receptors, including transferrin receptor, insulin receptor, LRP, LDL and TMEM30A (FC5 antigen) as well as a significant portion of released FC5, suggesting that exocytosis of BBB-crossing antibodies could occur via shedding exosomes.

The results imply that the presence of Fc fragment as well as a bi-valent target engagement by the BBB-crossing sdAbFC5 directs its intracellular sorting away from lysosomes and into early (and likely recycling) endosomes, resulting in facilitated transcytosis and release at the abluminal side of the BBB model. The implications of these findings for engineering BBB-crossing enzyme-replacement therapies will be discussed.

## PROGRANULIN EXPRESSION AND FRONTOTEMPORAL DEMENTIA (FTD), A LINK WITH NEURONAL CEROID LIPOFUSCINOSIS (NCL)

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The progranulin protein is a growth factor that also possesses anti-inflammatory properties. In the brain, it is widely expressed in neurons and activated microglia. Heterozygous loss-of-function mutations of progranulin (GRN) lead to low levels of the progranulin protein and are implicated in one form of frontotemporal dementia (FTD), an early onset neurodegenerative disease. FTD, the most common cause of dementia in patients under age 65, has several distinct clinical variants. Patients with mutations in GRN most commonly present with profound disturbances in personality, social function, judgement and insight; a syndrome termed behavioural variant FTD. Alternatively, some mutation carriers may present with progressive non-fluent aphasia, which is characterized by hesitant, effortful speech and articulatory problems. A subset of progranulin mutation carriers also exhibit Parkinsonism. Biochemically, progranulin-deficient FTD is distinguished by ubiquitin-rich and TAR DNA-binding protein 43 (TDP-43)-positive inclusions visible upon brain autopsy. FTD is currently untreatable; patients usually progress to limited function and die within a few years after diagnosis.

While FTD is caused by a mutation in one copy of the progranulin gene, recently two individuals were identified with mutations in both copies of the progranulin gene. These homozygous null GRN subjects presented with adult onset neuronal ceroid lipofuscinosis (NCL), displayed negligible circulating progranulin levels, and exhibited symptoms including seizures and retinal atrophy. Thus, progranulin gene dosage determines whether a patient develops FTD or NCL and suggests these two diseases, previously thought to be distinct, are highly related. Specifically, because NCL is a lysosomal storage disorder, this discovery implied progranulin must be required for proper lysosomal function. Indeed, murine models of both the heterozygous and homozygous Grn forms have been studied and recapitulate some of the pathophysiological phenotypes observed in humans with FTD or NCL. In Grn-deficient mice, retinal thinning and lipofuscin-rich deposits were observed as early indicators of disease. This signal was confirmed in human studies of progranulin mutation carriers and may provide a useful non-invasive diagnostic for disease.

The Bluefield Project to Cure Frontotemporal Dementia is a non-profit foundation dedicated to finding a cure for progranulin-deficient frontotemporal dementia. We fund basic, translational and clinical research at 14 institutions across North America. In this presentation, we will describe our research model, recent discoveries in the biology of progranulin-deficient FTD, and our current therapeutic strategies.

## DRUG TARGETING TO THE BRAIN BY COLLOIDAL CARRIER SYSTEMS

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Most drugs, particularly biologicals, do not reach the central nervous system, because they are not able to cross the blood – brain barrier, which is formed by brain capillary endothelial cells. These cells are connected to each other by extremely tight junctions and in addition they are equipped with a battery of potent ABC (ATP binding cassette) - export proteins like p-glycoprotein or breast cancer resistance protein, recognizing a multitude of completely diverse substrates and thus making effective drug delivery to the CNS extremely difficult. Here, an overview on the use of colloidal carriers to overcome the BBB will be given. Several technical strategies have been exploited to deliver macromolecular drugs to the brain, e.g., the use of vector-coupled liposomes or surface modified nanoparticles consisting of biodegradable polymers or lipids. They are promising delivery systems due to their potential in encapsulating drugs, their ability to escape p-glycoprotein in the blood brain barrier and to target the brain. E.g., nonviral gene transfer (plasmids encoding either luciferase or  $\beta$ -galactosidase) to primate brain was demonstrated after encapsulation into PEGylated immunoliposomes, which had been coupled to a monoclonal antibody to the human insulin receptor. The level of luciferase gene expression in the brain was 50-fold higher in rhesus monkey as compared to rat and neuronal expression of the  $\beta$ -galactosidase gene in brain was demonstrated by histochemistry and confocal microscopy. Polysorbate-80 coated and drug loaded poly(alkyl-acrylate) nanoparticles have been shown to effectively cross the BBB and to distribute in brain tissue. It has been proposed that apolipo-proteins get adsorbed on the surface of the nanoparticles in human plasma. Such nanoparticles mimic lipoprotein particles and thus may be endocytosed via a lipoprotein receptor-mediated mechanism. The permeation of nanoparticles across the blood brain barrier can be visualized by fluorescence labeling and subsequent confocal laser scanning microscopy [1]. These studies give clear evidence of a localization of particle-associated fluorescence within microvessel endothelial cells as well as beyond the brain capillaries. An aspect of concern may be the safety of these particles. In vitro studies indicate a transient decrease of transendothelial resistance and in vitro as well as in vivo experiments show a significant release of certain cytokines in blood after i.v. administration suggesting that careful monitoring of potential inflammatory events might be necessary. Similar results as with polymeric nanoparticles can be obtained with solid lipid nanoparticles consisting of lipids such as cetyl palmitate, Dynasan 114, Witexsol E85 and surfactants like Polysorbates 20, 40, 60, or 80.

The present data indicate that colloidal polymeric systems represent a promising strategy to overcome the blood brain barrier. However, further efforts are required to clarify in more detail the fate of the polymer after drug release as well as clinical efficacy of the used systems.

## DRUG DELIVERY TO THE CNS BY POLYMERIC NANOPARTICLES: WHAT DO WE KNOW?

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The blood-brain barrier (BBB) represents an insurmountable obstacle for the delivery of a large number of drugs to the central nervous system (CNS). One of the possibilities to overcome this barrier is the drug delivery to the brain using nanoparticles. The pharmaceutical and medical definition of nanoparticles is: Nanoparticles are solid polymeric particles of a size between 10 and 1000 nm into which drugs or bio-logically active materials are incorporated, surface adsorbed or chemically bound. The paramount requirement for the suitability of nanoparticle core polymers for brain delivery is that they are biocompatible and rapidly biodegradable. For this reason, presently only poly(alkyl cyanoacrylates), polylactides, and crosslinked human serum albumin (HSA) can be used as nanoparticle materials for brain delivery.

Drugs that have been transported into the brain and led to a pharmacological effect after intravenous injection using this carrier include the hexapeptide dalargin, the dipeptide kyotorphin, loperamide, tubocurarine, doxorubicin, the NMDA receptor antagonists MRZ 2/576 and MRZ 2/596, and others<sup>1</sup>. To achieve a significant transport across the blood-brain barrier the coating of the nanoparticles with polysorbate 80 (Tween® 80) was a key factor<sup>1,2</sup>.

Experiments with the extremely aggressive glioblastoma 101/8 transplanted intracranially showed a long term survival for 6 months of up to 40 % of the rats after intravenous injection of the polysorbate 80-coated nanoparticle preparation. Histological investigation of the surviving animals showed a total remission. Untreated controls died within 10 - 20 days, the animals in the doxorubicin control and uncoated doxorubicin nanoparticle groups died between 10 – 50 days.<sup>2-4</sup>

The cardiac, and testicular toxicity of doxorubicin was very significantly reduced by binding the drug to the poly(butyl cyanoacrylate) nanoparticles. In addition the haematological and liver toxicity could be reduced. Coating of PBCA nanoparticles with polysorbate 80 contributed to the reduction of the toxicity. The lower toxicity of the nanoparticle formulations can be most probably explained by the altered biodistribution of the drug mediated by the nanoparticles<sup>7-9</sup>. Indication of short-term neurotoxicity, such as increased apoptosis in areas distant from the tumor, increased expression of GFAP or ezrin on distant astrocytes or degenerative morphological changes of neurons were entirely absent in the rats on day 12 as well as in long-term survivors<sup>3,4</sup>.

The polysorbate 80-overcoated poly(butyl cyanoacrylate) nanoparticles also enabled the delivery of nerve growth factor (NGF, MW ~ 130 kDa) into the brain of outbred and of C57BL/6 mice after intravenous injection<sup>10</sup>.

The nanoparticles yielded considerably enhanced NGF levels in the brain and induced pronounced and prolonged pharmacological effects: The nanoparticles with bound NGF were able to totally reverse the scopolamine-induced amnesia and improved the recognition and memory in an acute amnesic mouse model. Moreover, in a number of Parkinson's disease models these particles significantly reduced the basic symptoms of Parkinsonism such as oligokinesia, rigidity, and tremor. The mechanism of the drug transport across the blood-brain barrier with the nanoparticles appears to be receptor-mediated endocytotic uptake by the brain capillary endothelial cells followed either by release of the drugs in these cells and diffusion into the brain or by transcytosis. After injection of the nanoparticles, apolipoproteins A-I or E adsorb on the particles surface promoting the interaction with receptors on the endothelial cells followed by endocytosis and thus mimic the uptake of naturally occurring lipoprotein particles. This hypothesis was supported by the achievement of an antinociceptive effect with loperamide-loaded albumin nanoparticles with covalently bound apo E and by electron microscopy.<sup>5,6</sup> Other targeting moieties that could be used for receptor-mediated brain capillary endothelial cell uptake include apolipoprotein A-1 or B, transferrin, insulin as well as antibodies against the respective receptors.

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## ANTI-INFLAMMATORY THERAPY WITH SIMVASTATIN IMPROVES THE CENTRAL NERVOUS SYSTEM DISEASE OF A DEMYELINATING MOUSE MODEL OF METACHROMATIC LEUKODYSTROPHY

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Metachromatic leukodystrophy (MLD) is a lysosomal storage disease caused by a functional deficiency of the lysosomal enzyme arylsulfatase A. The prevailing late-infantile variant of MLD is characterized by widespread and progressive demyelination of the CNS causing death during childhood. To get insight into the pathomechanism of the disease and to identify novel therapeutic targets, we analysed neuroinflammation in two mouse models reproducing a mild, non-demyelinating, and a more severe, demyelinating, variant of MLD, respectively. Microgliosis and upregulation of cytokine/chemokine levels were clearly more pronounced in the demyelinating model. The analysis of the temporal cytokine/chemokine profiles revealed that the onset of demyelination is preceded by a sustained elevation of the macrophage inflammatory protein (MIP)-1 $\alpha$  followed by an upregulation of MIP-1 $\beta$ , monocyte chemoattractant protein (MCP)-1 and several interleukins. The tumor necrosis factor (TNF)- $\alpha$  remains unchanged. Treatment of the demyelinating mouse model with the non-steroidal anti-inflammatory drug simvastatin reduced neuroinflammation, improved the swimming performance and ataxic gait and retarded demyelination of the spinal cord. Simvastatin treatment had no effect on cholesterol levels and sulfatide storage in the CNS. Our data demonstrate that neuroinflammation is causative for demyelination in MLD mice and suggest that anti-inflammatory treatment might be a novel therapeutic option to improve the CNS function of MLD patients in preclinical and clinical stages of the disease.

## EXPERIENCE WITH INTRACEREBROVENTRICULAR DELIVERY OF A LYSOSOMAL ENZYME IN THE CLINICAL TRIAL FOR CLN2 DISEASE

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CLN2 disease (classical late-infantile ceroid lipofuscinosis, Janský-Bielschowsky Disease, Omim 204500) is caused by the genetic deficiency of the lysosomal enzyme tripeptidyl peptidase 1 (TPP1), the natural substrate of which is unknown. The enzyme defect causes intralysosomal accumulation of storage material and loss of neurons as well as of retinal cells, while the chain of events uniting the different processes remains to be established. Affected children usually have a normal psychomotor development until their third year of life, sometimes their acquisition of language is slightly retarded. The disease manifests itself with developmental standstill or epilepsy and subsequent rapid loss of all psychomotor abilities. Supplying artificially produced TPP1 to the CNS of a natural dog model of CLN2 disease via infusion into the CSF space has led to wide-spread distribution of the active enzyme in the brain, to reduced abnormal cellular storage and to functional improvement. Because of the positive results of preclinical studies, a phase I/II trial of enzyme replacement to the brain of children with CLN2 disease was started in September 2013. A direct access to a lateral brain ventricle was chosen, using an Ommaya reservoir that is repeatedly punctured through the scalp. A total of 21 patients have been enrolled at four trial sites, of which 11 patients are being treated in Hamburg. We present our experience with the trial after 14 months of treatment. The functionality of the system of enzyme replacement and the tolerance of the procedure were good, while the clinical efficacy will be determined on the basis of historical quantitative clinical data later in 2015. Diagnosis of CLN2 disease has become easy as a dry blood spot test for TPP1 activity is available, shortly to be replaced by a multiplex test for several lysosomal storage disorders. Slow language acquisition in an otherwise healthy-appearing infant may put the child at risk for being a future CLN2 patient.

## INVESTIGATIONAL INTRATHECAL (IT) ENZYME REPLACEMENT THERAPY FOR THE SEVERE FORM OF HUNTER SYNDROME

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Hunter syndrome (Mucopolysaccharidosis II, MPS II) is an X-linked lysosomal storage disorder caused by a deficiency in the enzyme iduronate-2-sulfatase, leading to the accumulation of glycosaminoglycans (GAG) in lysosomes. About one-third of patients have intact cognition (attenuated phenotype) and about two-thirds experience progressive cognitive impairment (severe phenotype) with death typically in the teenage years. Idursulfase (Elaprase®, Shire, Lexington, MA, USA), a recombinant human iduronate-2-sulfatase administered intravenously, does not cross the blood-brain barrier at the recommended therapeutic dose and is not expected to alter the cognitive decline in MPS II patients with a severe phenotype. In preclinical animal studies, intrathecally administered idursulfase penetrated into brain tissue, was taken up by cells in the CNS, and was capable of reducing lysosomal GAG storage. A six month multicenter, open-label, multiple-dose phase I/II study (HGT-HIT-045; NCT00920647) to determine the safety and tolerability of ascending-dose regimens of an investigational formulation of idursulfase designed for intrathecal (IT) administration (idursulfase-IT) in patients with severe MPS II was conducted. Idursulfase-IT was administered monthly via a surgically implanted IT drug delivery device (IDDD) or via lumbar puncture. After completion of the initial study, all eligible patients enrolled in a long-term extension study (HGT-HIT-046, NCT01506141). There are currently 13 patients in the extension study. The longest follow-up is currently 60 months. The safety, pharmacokinetic, cerebrospinal fluid GAG response, and cognitive outcome data from HGT-HIT-045 and HGT-HIT-046 will be presented.

*Shire (Lexington, MA) sponsored the clinical trials.*

## TRANSITION OF ANTIBODY DIRECTED RECEPTOR MEDIATED TRANSCYTOSIS THERAPIES INTO CLINICAL STUDIES

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The biotechnology industry has become increasingly interested in employing antibodies against receptors on the BBB to deliver complex biologics into the CNS. We have targeted the endogenous BBB receptor-mediated transport system of the human insulin receptor (HIR) with a monoclonal antibody (MAb) re-engineered with enzyme fusions to treat mucopolysaccharidoses. The HIRMAb domain of these fusion proteins act as a molecular Trojan horse to ferry fused enzymes iduronidase (IDUA) and iduronate 2 sulfatase (IDS) across the BBB. Data will be presented confirming full enzyme activity for both constructs, resulting in reversal of pathology within the CNS in murine models and facilitates differential transport across the BBB in Rhesus monkeys as compared to current therapy. In addition, we will report on the data from chronic nonclinical safety studies demonstrating a wide therapeutic window to support initiation of our clinical studies. Our near-term clinical development and pipeline plans will also be presented. This work further advances the theory that re-engineering of biotherapeutics enables safe and rapid BBB penetration for treatment of the neurological sequelae in a range of disorders.

## LYSOSOMAL MULTIENZYME COMPLEX: PROS AND CONS OF WORKING TOGETHER

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In lysosomes the degradation or processing of macromolecular substrates requires the synergistic activity of multiple hydrolases that normally operate in a stepwise fashion. This catalytic machinery works optimally if the enzymes assemble in complexes of 2 or more proteins. This configuration allows for a rapid and dynamic response of the enzymes within the complex to changes in substrate composition/concentration and, hence, in the metabolic needs of functionally different cell types. One such complex is composed of the serine carboxypeptidase, protective protein/cathepsin A (PPCA), the sialidase, neuraminidase-1 (NEU1), and the glycosidase  $\beta$ -galactosidase ( $\beta$ -GAL). In complex these enzymes contribute to the catabolism of numerous glycoconjugate substrates, many of which still unknown, and, thereby, maintain cell and tissue homeostasis. On the other hand, genetic mutations that affect one of the three enzymes in the complex indirectly influence the properties and functions of the other two, and give rise to the multi-systemic phenotypes characteristic of three neurodegenerative lysosomal storage diseases (LSDs): GM1-gangliosidosis ( $\beta$ -GAL deficiency), galactosialidosis (PPCA deficiency), and sialidosis (NEU1 deficiency). Studies of the mechanisms of pathogenesis in the mouse models of these LSDs have uncovered basic cellular processes that are directly controlled by the multi-enzyme complex and its substrates. In addition, they have identified important connections between these rare pediatric diseases and more common neurodegenerative conditions, like Alzheimer's disease, normally occurring in the aging population. A deeper understanding of the cell biology and pathogenesis of pediatric LSDs may ultimately lead to the development of novel therapies for a much broader number of patients than those affected by these disorders.

## PRESENTATION OF THE INHERITED NEUROMETABOLIC DISEASES INFORMATION NETWORK (InNerMeD-I-Network) AND WEBSITE PLATFORM TO INCREASE AWARENESS ON NEUROMETABOLIC DISEASES

### **MAURIZIO SCARPA<sup>1</sup> AND FEDELE (DUCCIO) BONIFAZI<sup>2</sup>, ON BEHALF OF ALL INNERMED PARTNERS**

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The Project "Inherited NeuRoMetabolic Disease Information Network" (InNerMeD-I-Network), is an European project 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 2012 12 12) to be the first European Information Network on Inherited NeuroMetabolic Diseases (iNMDs). It started on the 1st of April 2013 but was officially kicked off in Luxemburg on May 21st, 2013.

Inherited NeuroMetabolic Disorders (iNMDs) are a group of rare genetic metabolic diseases that impact on the brain causing mental retardation and progressive neurodegeneration which, if not promptly treated, could end in early death. Lack of information on these conditions can lead to delayed diagnosis and treatment, with consequent tragic results. Increasing awareness is therefore the first crucial step in fighting these pathologies.

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 communities, 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 treatments (experimental and marketed). 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 (Italy), includes 4 associated partners: Gianni Benzi Pharmacology Research Foundation (Italy), Copenhagen University Hospital (Denmark), University of Zagreb (Croatia) and Hospital Sant Joan de Déu (Spain), plus

fifteen collaborating partners including clinical and research centres, patients and parents associations and scientific societies.

Thanks to partner's specific expertise, InNerMeD-I-Network will create a formidable concentration of competences in such a complex and heterogeneous medical field.

Here we will present the IT platform which is functional to the project itself as it constitutes the main tool for reducing the knowledge gap in the fields of iNMDs. It is, in fact, aimed at connecting all the network nodes and developing main networking activities with the plurality of external stakeholders. Most of all, it will host the InNerMeD-I-Network Database, containing information targeted on research, diagnosis and treatment of iNMDs, a key instrument for increasing awareness on neurometabolic diseases and reducing the fragmentation of information in the fields of iNMDs.

## MODELLING AND SIMULATION, EXTRAPOLATION AND ALTERNATIVE STUDY DESIGNS AS INNOVATIVE APPROACHES TO DEVELOP PAEDIATRIC DRUGS FOR INMDS

### GIOVANNI SMANIA

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The design and implementation of paediatric trials are challenging and often difficult to accomplish. Ethical, practical and even financial considerations have caused the evaluation of efficacy and safety of drugs in children to be based on empirical extrapolations from clinical trials in adults. Moreover, such challenges are exacerbated in NeuroMetabolic Diseases (NMDs), since they are classified as rare diseases.

The design, analysis and interpretation of clinical studies in this particular subset of the paediatric population require specific techniques to ensure accurate decision-making regarding the pharmacokinetics, safety and efficacy of drugs, as also supported by the guideline on clinical trials in small populations set by the European Medicine Agency (EMA), which states that “crude (simple) methods may often be adequate when we have huge amounts of data, but when there are very few data, it is imperative that the most efficient and informative analytical methods should be used” [1].

Extrapolation is a strategic approach that may allow one to circumvent some of the aforementioned difficulties: it consists in extending information and conclusions available from studies in one or more subgroups of the patient population (source population), or in related conditions or with related medicinal products, to make inferences for another subgroup of the population (target population), or condition or product, thus reducing the need to generate additional information [2].

A very useful methodological tool that naturally fits into the context of extending information from a source population to make inferences for another population is Modelling and Simulation (M&S). The added value of M&S in paediatric clinical research has been extensively documented [3], and its weight at a regulatory level in supporting extrapolation has constantly been increasing in the last years. However, extrapolation is a much broader clinical decision process than making calculations using a model and should not be used as a tool for replacement of clinical studies, especially when dealing with diseases specific to children such as NMDs. Nonetheless, even when extrapolation cannot be applied to its full extent, its use is still highly needed in order to optimize the generation of new data through ad-

ditional clinical studies. In fact, extrapolation is of paramount importance in defining the best starting dose and in designing the new trial by means of innovative study designs (such as Bayesian and sequential approaches), which ultimately allow to reduce the number of patients required, making these techniques appealing, or rather compulsory, for the development of new paediatric drugs for NMDs.

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## IMPACT OF THE NEW EUROPEAN CLINICAL TRIAL REGULATION ON INMDS PAEDIATRIC RESEARCH

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So far, limits in paediatric clinical trial (CT) rules have been highlighted, due to the different implementation of the Directive 2001/20/EC, and to the divergences in the existing guidelines, including ICH Topic E11, and the Ethical Recommendations on paediatric trials issued by the EC in 2008.

The European framework surrounding CTs is going to change, as demonstrated by the large debate had at European institutional level, which has led to the release of the new EU CT Regulation (EU) 536/2014. One of the major challenge is to overcome the lack of harmonisation of CT procedures among countries. This aspect gains more and more importance in paediatrics and rare diseases, also considering that in these fields studies are typically multi-centre and multi-national.

Analysing the rules introduced by the new EU CT Regulation (EU) 536/2014 and covering all stages (including the preparation of documents, the Clinical Trial Application – CTA -, the conduction of the study), some issues favouring the conduction of multi-centre CTs (such as the centralisation of CTs assessment at national level, a well-defined list of documents to be submitted and their contents) have been introduced.

In addition, in the first draft of the new EU CT Regulation released by the EC, the most recent provisions established in the above mentioned EC Paediatric Recommendations were lacking, but in the final version some concepts have been included: the involvement of minors in the informed consent procedure according their age and mental maturity, and the need for paediatric expertise or advice in Ethics Committees.

Unclear aspects deal with the definitions of benefit/risk, minimal risk-minimal burden and “low-interventional trials”. Unfortunately, while recognising the importance of clinical trials for the development of orphan drugs and of a timely availability of drugs for rare and ultra-rare patients, no major improvement has been reached in the field of rare diseases. In fact, the Regulation does not establish mandatorily a more rapid assessment and the involvement of specific expertise when assessing clinical trials involving these patients.

In conclusion, the new Regulation introduces rules that will harmonise the clinical research in EU, affecting positively the paediatric one. Furthermore, a stronger rule, such as a Regulation, will make mandatory some important preconditions, already stated in previous non-mandatory documents, to start a trial.

However, the standardisation of procedures among EU countries will rely on the implementation of the new rules at national level. A close collaboration between Member States and the main stakeholders should be the only way to reach this goal.

## INNOVATION AT SHIRE – DRUG DISCOVERY APPROACH FOR CENTRAL NERVOUS SYSTEM TARGETING

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There are approximately 7000 known rare and orphan diseases, with a combined prevalence of 25 million in the US and 25-30 million in Europe. Despite over a third of these affecting the central nervous system, treating the pathologies caused by lysosomal storage disorders and other rare neurological diseases remains an area of urgent unmet medical need. Shire is committed to developing innovative specialty medicines to address significant unmet medical needs by selecting the right biological target and the right delivery mechanism based on extensive knowledge of disease pathophysiology and draws on the array of technology platforms available at Shire including antibodies, modified RNA, small molecules, gene therapy and protein therapeutics. Importantly, Shire leverages experience gained from both the pre-clinical and clinical paths of the intrathecal enzyme replacement therapy delivery program to guide the exploration of less invasive delivery modalities. For example, Shire recently formed a partnership with ArmaGen to test a specific compound for the central nervous system and somatic manifestation of Hunter Syndrome. New targets and technologies are constantly under evaluation and will hopefully lead to effective and minimally invasive next-generation strategies for central nervous system drug delivery.

## PLASMA LYSPHINGOLIPIDS AS BIOMARKERS FOR NIEMANN-PICK DISEASE TYPE C: USE IN DIAGNOSE AND POTENTIAL FURTHER APPLICATIONS

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Niemann-Pick disease type C (NP-C) is a devastating, neurovisceral lysosomal storage disorder that affects children, adolescents and adults. NP-C is caused by mutations in the NPC1 and NPC2 genes and is characterised clinically by heterogeneous manifestations of visceral signs, progressive neuropsychiatric deterioration and early death [1]. Due to the difficulty of diagnosis and the availability of an approved therapy in the EU, improved detection of NP-C may positively impact future disease management. At the cellular level, dysfunction or deficiency of either the NPC1 or NPC2 protein leads to an intracellular endosomal/lysosomal trafficking defect resulting in lysosomal accumulation of unesterified cholesterol and organ specific patterns of sphingolipid accumulation. Plasma lysosphingolipids have recently been shown to be excellent biomarkers of sphingolipidosis in the enzyme deficient lysosomal storage disorders Fabry disease [2] and Gaucher disease [3]. In a recent prospective study of adults with neurological and psychiatric symptoms the lysosphingolipids, lysosphingomyelin (SPC) and glucosylsphingosine (GlcSph), appeared to be elevated in the plasma of three newly diagnosed NP-C patients [4]. In order to investigate the clinical utility of plasma SPC and GlcSph as diagnostic markers for NP-C, a liquid chromatography-tandem mass spectrometry assay was validated for their measurement. Plasma SPC and GlcSph can be measured accurately, precisely and reproducibly and are stable in both plasma and whole blood. In a retrospective study of 57 NP-C patients and 70 control subjects, median plasma SPC and GlcSph were significantly increased in NP-C by 2.8-fold and 1.4-fold respectively [5]. The elevation of both markers was inde-

pendent of age, indicating that the increase is unlikely to be linked to a single NP-C visceral symptom. Treatment with the glucosylceramide synthase inhibitor miglustat did not appear to significantly alter plasma SPC, while there was a strong trend for decreased GlcSph in treated patients. Plasma SPC did not correlate with either GlcSph or cholestan-3b,5a,6b-triol levels in NP-C patients, indicating multiple markers would likely give the best sensitivity for NP-C diagnosis. For miglustat-naïve NP-C patients, aged 2–50 years, the area under the ROC curve was 0.999 for SPC, indicative of utility as a NP-C diagnostic marker, where it could be used to identify NP-C patients before confirmatory sequencing.

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## USING A PEPTIDE DERIVED FROM TRANSCEND (MTf, P97) TO DELIVER BIOLOGICS TO THE CNS USING A PHYSIOLOGIC PATHWAY

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biOasis Technologies Inc. is a ground-breaking biopharmaceutical company focused on the delivery of therapeutics across the blood-brain barrier and into the brain tissue. The Company is developing proprietary peptide vectors based on melanotransferrin (MTf) for the delivery of therapeutics to the CNS, this platform is called “Transcend”.

The delivery of therapeutics across the blood-brain barrier (BBB), represents the single greatest challenge in the treatment of over a thousand common and rare diseases of the central nervous system. The BBB is formed by brain capillary endothelial cells, which are closely sealed by tight junctions and express high levels of active efflux transport proteins. Specific receptors and transport systems are highly expressed at the BBB to provide essential substances to brain cells. These important characteristics provide a natural defense against toxic or infective agents circulating in the blood. Therefore, the development of new technology 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 protein (Melanotransferrin or MTf, p97) and peptides thereof, have been developed by biOasis Technologies Inc. and are used to facilitate receptor mediated drug delivery into the brain to treat CNS disorders.

Using antibodies and lysosomal enzymes labeled with fluorescent dyes we demonstrated that: antibodies against Her2 (Traztuzumab, TZM); against BA1-42 peptides (6E10); lysosomal enzymes such as α-L-iduronidase (IDU) or iduronate-2-sulfatase (I2S), are transported at therapeutic concentration across the BBB in brain cells after conjugation to Transcend. Transcend conjugates are rapidly and efficiently transported in the brain parenchyma and in the lysosomal compartment of neurons and astrocytes.

Using laser scanning confocal microscopy, an increase of approximately 10 times of the distribution of BT2111 in the brain parenchyma compared to TZM was observed 2 hr post-IV injection. Therapeutic efficacy was demonstrated using a mice model characterized by the formation of brain metastasis after in-

tracardiac administration of MDA-MB 231BR we show efficacy of BT2111 in decreasing the amount and size of brain metastasis in this mice model. It was found that BT2111 reduced the number of human HER2+ breast cancer metastases in the brain by 68% when compared to control animals. The tumours that remained after treatment were 57% smaller than those in controls, equating to an overall 86% reduction in tumour volume. In contrast, TZM alone had no effect.

The family of peptides that we identified from MTf have shown to be efficiently transported across an in-vitro BBB model as well as in vivo, both demonstrating a high transcytosis rate. The lead peptide has shown very efficient and rapid transport across the BBB and was able to increase significantly the delivery of an antibody to the CNS after its chemical incorporation or expressed in a fusion protein. The application of this new peptide vector to oligonucleotides such as siRNA and on-going studies addressing the brain delivery of I2S for the treatment of Hunter Syndrome in k/o mice will be discussed.

These studies will provide the proof of concept that Transcend both full length MTf and its derived peptides, can be used as carriers capable of shuttling a variety of compounds ranging from small anti-cancer agent to larger biologics across the BBB into the brain parenchyma in therapeutic doses that enable treatment of neurological disorders.







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