BRAINS FOR BRAIN

European Task Force on Brain and Neurodegenerative Lysosomal Storage Diseases

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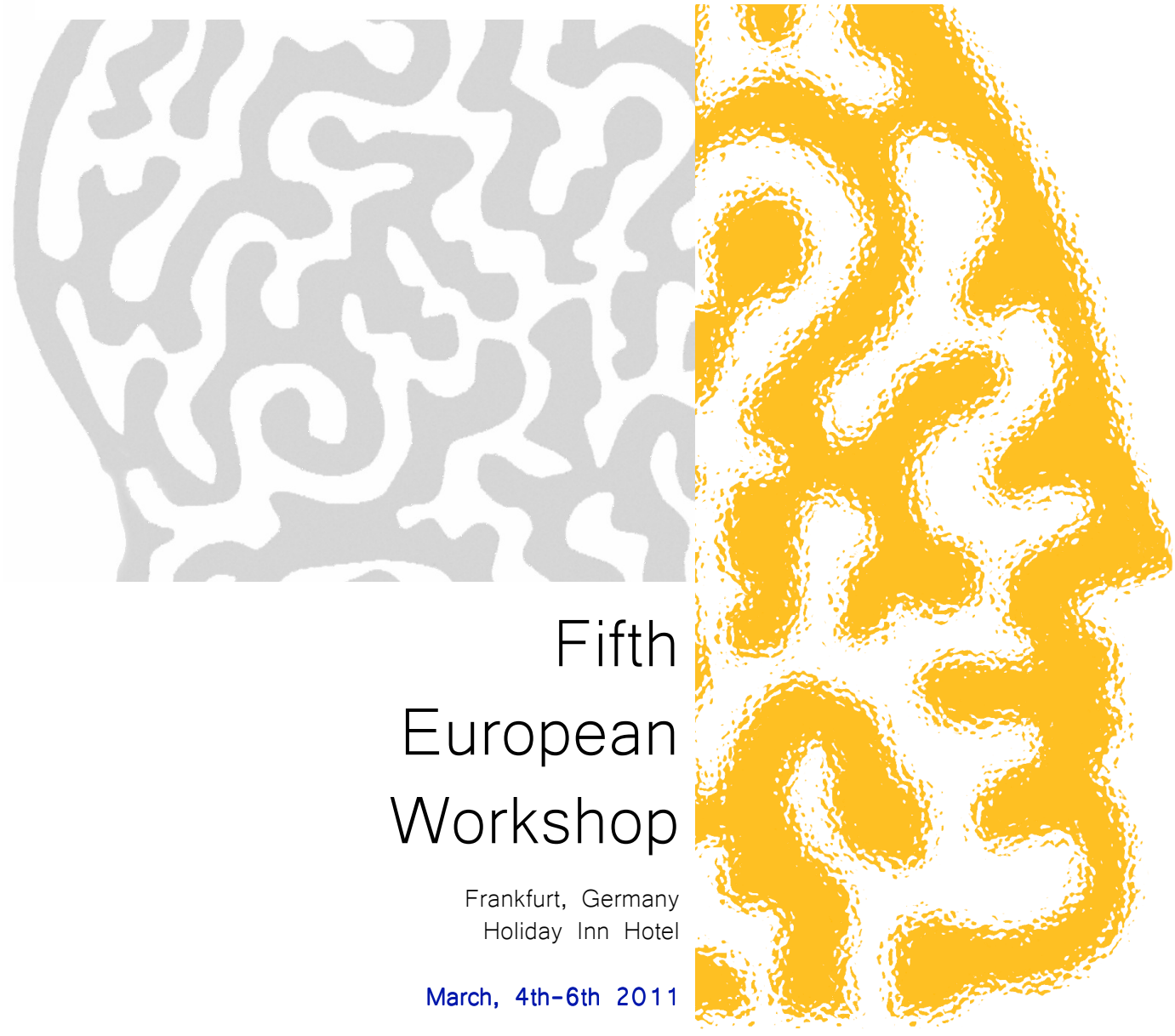

Fifth European Workshop

Frankfurt, Germany
Holiday Inn Hotel

March, 4th-6th 2011

SCIENTIFIC PROGRAMME 2011

March 4th 2011

14.00-14.10
WELCOME AND OPENING
14.15-15.00
Opening Plenary Lecture
PAUL SAFTIG, DE
New insights into lysosomal function.
University of Kiel.
Discussion.
15.00-19.15

BASIC ASPECTS AND BBB

Chair Discussants:
GERT FRICKER, DE - INGOLF BLASIG, DE
15.00-15.30
HELEN STOLP, UK
Effects of environmental influences on foetal cortex and vascular development.
University of Oxford.
Discussion.
15.40-16.10
BRITTA ENGELHARDT, CH
Migration of immune cells across the BBB.
Theodor Kocher Institute, Berne.
Discussion.
16.20-16.50
GIOVANNA DEL VECCHIO, IT
High throughput screening for claudin 5 modulators to manipulate the BBB.
Liebnitz Institute of Molecular Pharmacology,Berlin.
Discussion.
Coffee
17.20-17.50
ELGA DE VRIES, NL
BBB and multiple sclerosis:
are there lessons for LSDs?
University of Amsterdam.
Discussion.
18.00-18.30
SANDRINE VITRY, FR
Cellular disorders in Mucopolysaccharidosis type III B.
Pasteur Institute, Paris.
Discussion.
18.40-19.10
ALESSANDRO FRALDI, IT
Cholesterol abnormalities and lysosomal dysfunction in LSDs.
TIGEM; Naples.
Discussion.
19.30 DINNER

March 5th 2011

PATHOPHYSIOLOGY AND LSDs

Chair Discussants:
ASHOK VELLODI, UK
VOLKMAR GIESELMANN, DE

9.00-9.30
MIA HORWITZ, IL
ER associated degradation and unfolded protein response in Gaucher disease.
University of Tel Aviv.
Discussion.

9.40-10.10
JON COOPER, UK
Batten disease.
Kings College, London.
Discussion.

10.20-10.45
EMYR LLOYD-EVANS, UK
Smith-Lemli-Opitz Syndrome; a recently identified lysosomal storage disease caused by defective cholesterol biosynthesis.
Cardiff University.
Discussion.

Coffee

CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 1

11.15-11.40
MARGARETA HAMMARLUND-UDENAES, SE
Drug delivery to the CNS.
University of Uppsala.
Discussion.

11.50-12.20
ROBERT KATONA, HU
A potential strategy for the treatment of neurological disorders: combined mammalian artificial chromosome-stem cell therapy.
Hungarian Academy of Sciences Szeged,Hungary
Discussion.

13.00 LUNCH

14.30-16.00

CROSSING THE BLOOD BRAIN BARRIER AND THERAPEUTICAL OPTIONS 2

Chairperson:
JEAN MICHEL HEARD, FR
JÖRG KREUTER, DE

14.30-15.00
JÖRG KREUTER, DE
On the mechanism of nanoparticle drug delivery across the Blood-Brain Barrier: transport kinetics and influence
of targeting igand attachment.
University of Frankfurt.
Discussion.

15.10-15.40
VOLKMAR GIESELMANN, DE
Transport of Arylsulfatase A across the Blood Brain Barrier.
University of Bonn.
Discussion.

15.50-16.20
ARI ZIMRAN, IL
OTC PC: From rats and type 1 Gaucher disease to patients with type 3 Gaucher disease: Give Ambroxol a chance!
Shaare Zedek Medical Center Jerusalem.
Coffee
17.00-17.30
BRIAN BIGGER, UK
The role of genistein in the treatment of MPSIII.
University of Manchester, UK.
Discussion.

17.40-18.10
SIMON WADDINGTON, UK
Perinatal gene therapy for lethal genetic diseases.
University College London, UK.
Discussion.
18.20-19.00
MARIE VANIER, FR
Plenary Lecture
Niemann-Pick C disease: the enigma and the challenges.
Inserm, FR.
Discussion.
20.30 DINNER

March 6th 2011

B4B AND EUROPE

Chair Discussants:
MAURIZIO SCARPA, IT - DAVID BEGLEY, UK
8.45-9.20
B4B and Europe Activity report of activity and perspectives.
9.20-9.45
OLGA GOLUBNITSCHAJA, DE
Asphyxiated newborns - common origin but individual outcomes: time for new guidelines in personalised healthcare
EPMA, Brussels
9.45-10.15
LARS KRISTIANSEN, FR
Introduction to the ESF Forward Look on personalised medicine.
ESF, EMRC, Strasbourg, France.
10.15-10.45
General discussion.
Coffee
11.00-13.30

B4B AND BIOTECH COLLABORATIONS

Chair Discussants:
CATHERINE CAILLAUD, FR
MIA HORWITZ, IL
11.00-11.20
PERICLES CALIAS, USA
Intrathecal (IT) delivery of recombinant lysosomal enzymes.
Shire Human Genetic Therapies.

Discussion.

11.30-11.50

REINHARD GABATHULER, CA

A new peptide vector (p97) for delivery of therapeutic compounds across the BBB for the treatment of brain diseases.

biOasis Technologies

Discussion.

12.00-12.20

SEAN CLARK, USA

Improved pharmacological chaperones for the treatment of neuronopathic Gaucher and Parkinson's disease.

Amicus Therapeutics.

Discussion.

12.30-12.50

THOMAS KIRKEGAARD JENSEN, DK

Hsp70 stabilizes lysosomes and reverts Niemann-Pick disease-associated pathology.

Orphazyme

Danish Cancer Society, Denmark

Discussion.

13.00-13.30 LUNCH AND FAREWELL

4th march 2011

Opening of the meeting

David and Maurizio officially opened the workshop by welcoming the participants and sharing some comments on B4B history and recent activities and shortly presenting the objectives of the meeting.

In the opening session, it was very important and stimulating to listen to the plenary lecture by **Dr. P. Saftig** (university of Kiel, DE) about new insights into lysosomal function.
He observed that lysosomes are the primary catabolic compartments of eukaryotic cells and are involved in various physiological processes, such as cholesterol homeostasis, antigen presentation, plasma membrane repair, bone and tissue remodeling, pathogen defense, cell death and cell signaling. He then focalized his attention to lysosomal membrane proteins. He explained that many human diseases are caused by mutations in genes encoding for lysosomal membrane proteins and, in his lab, he is actually performing a functional analysis of “new“ lysosomal membrane proteins as the lysosome-associated membrane proteins (LAMP)-1 and -2 and the lysosomal integral membrane protein (LIMP)-2 which represents a newly discovered importer transport receptor to the lysosome and the tetraspanin CD63. The presentation provided clear and comprehensive overview about the roles of different lysosomal proteins in health and disease.

Scientific Session: Basic Aspects and BBB

In the opening session, speakers discussed about LSDs basic aspects and complexity of the brain barrier systems. Participants gave an overview about the new recent advances on biomolecular mechanism responsible of LSDs pathophysiology with main interest in CNS neurodegeneration and mechanisms for drug delivery to the brain and related therapeutic possibilities. It has been emphasized the fundamental function of BBB as a dynamic regulatory interface between the blood and brain. The BBB in fact, maintains the brain internal fluid environment extremely stable, and represents a protective barrier which shields the CNS from any neurotoxic substances which circulate in the blood but at the same time it severely limits the penetration of many drugs and therapeutic agents into the CNS. The session debated new insight morphological and functional mechanism that restricts or facilitates the passage of substances from blood to brain. The reported studies considered the BBB as a dynamic system integrated with both the CNS and the periphery. Various methodological approaches were proposed. These include measurement of transport between blood and brain, imaging-based technologies, and investigations of adhesion receptors on

BBB cells and their interaction with counter receptors on blood-borne cells, genomic/proteomic approaches. The BBB function in the context of disease was also considered. Improving our knowledge on BBB structure and pathophysiology could help us to overcome the difficulty of delivering therapeutic agents to specific regions of the brain and therefore presents a major challenge to treatment of most brain disorders. Up to date, in fact, therapeutic molecules and genes that might otherwise be effective in diagnosis and therapy do not cross the BBB in adequate amounts.

The first lecture for the day was given by **Dr. H. Stolp**, who talked about "Effect of environmental influences on foetal cortical and vascular development". She particularly focalized her attention on inflammation and brain development explaining that neurological disorders have been associated with inflammation during foetal development and animal studies show long term effects on the behaviour of the offspring. To date pathological changes in the brain as a result of inflammation, particularly during early brain development, are still largely unstudied. Therefore she reported data from her research study performed in mice model showing that that maternal inflammation causes reduced proliferation in the ventricular zone of the foetal cortex. She in fact evaluated the structural integrity of the foetal brain finding that the integrity of the ventricular surface was disrupted, but not the permeability of the blood-brain barrier. Such results support the hypothesis that prenatal inflammation plays a role in the development of neurological disorders.

Followed then the talk of **Dr. B. Engelhardt** who gave a talk about "Migration of immune cells across the BBB". She underlined that central nervous system (CNS) homeostasis is a prerequisite for the proper communication of neuronal cells. To this end, the endothelial blood-brain barrier (BBB) and the epithelial blood-cerebrospinal fluid barrier (BCSFB) tightly seal off the CNS from the continuously changing environment within the blood stream. She nicely explained and described the multistep cascade model throughout which leucocytes (memory T cells) can cross the non-inflamed BBB or BCSFB and cause experimental immune response. Leucocytes can in fact gain access to the cerebrospinal fluid (CSF) drained ventricular, subarachnoidal and perivascular spaces. Once crossed the BBB, if T cells encounter their specific antigen on antigen presenting cells strategically localized immediately behind the brain barriers, reactivation of the T cells will trigger a local inflammatory response leading to the stimulation of the BBB. As a consequence novel traffic signals determines localization of circulating inflammatory cells into the perivascular spaces and then across the glia limitans into the CNS parenchyma, where they progress to initiate tissue injury.

The scientific session followed then with the talk of **Dr. G. Dal Vecchio** entitled "High throughput screening for claudin-5 modulators to manipulate the BBB". She observed that BBB is formed by endothelial cells sealed at the level of the paracellular cleft by tight junctions (TJ). BBB limits the therapy of many CNS diseases and it is involved in CNS diseases as it has been showed that it is directly or indirectly compromised in many brain disorders, for example many CNS diseases are associated to uncontrolled BBB opening. Aim of her research is to investigate the possibility of modulating the BBB permeability acting on Claudin-5 (Cld5) leading to an enhancement or reduction of paracellular permeability in a cell selective manner. As a consequence drug delivery efficacy could be improved and some pathological conditions could be ameliorated by the re-establishment of normal barrier properties. She then described Cell-based High Throughput Screening (HTS) approach to target Cld5 and identify small-molecule modulators of the BBB. She concluded her talk underling that the assay targeting Cld5 to identify potential BBB opener and protective compounds has been successfully implemented and adapted to the HTS technology.

Followed the presentation of **Dr. E. De Vries** entitled **"**The Blood-Brain Barrier and Multiple Sclerosis (MS): are there lessons for LSDs? She explained that MS, a chronic inflammatory disorder of the central nervous system, is characterized by accumulation of inflammatory cells in the brain causing active lesions. Among inflammatory cells macrophages play a major role in the pathogenesis of MS due to the fact that they can phagocyte myelin and thereby induce demyelization. She underlined that to data there are only few data about the molecular properties of the BBB under neuroinflammatory conditions and so she decided to analyze well-defined post-mortem MS patient material: a dominant loss of P-glycoprotein (P-gp: ABCB1, the key ABC transporter which effectively removes a remarkably wide variety of substrates out of the brain, including inflammatory agents) was detected in active MS lesions. Strikingly, in MS lesions, reactive astrocytes start to express a number of these ABC transporters including multi-drug resistance protein-1 (MRP-1) and P-gp. She therefore suggested that an altered interplay between activated astrocytes and the brain endothelium could represent the first step toward BBB dysfunction in MS, thereby allowing MS lesion formation.

Dr. S. Vitry presented her work entitled "cellular disorders in Mucopolysaccharidosis Type III B (MPSIIIB)". She briefly described the MPSIIIB also known as sanfilippo syndrome type 3b (four biochemically distinct but clinically similar disorders all characterised by a deficiency in a lysosomal enzyme implicated in the degradation of heparan sulphate. In MPSIIIB cells, a deficiency in NaGlu is responsible for the accumulation of undegraded HSO. Little is known about the toxicity of HSO accumulation on these processes. She explained that her research aims at understanding cell biology disorders in neurons accumulating non degraded HSO by mean of mouse model of

MPSIIIB, new models of the disease using iPSc derived from skin fibroblasts of affected children and HeLa cells in which NaGlu, the missing enzyme in MPSIIIB, can be depleted by tetracycline-inducible shRNAS and applied gene expression profiles analysis. Data suggest that HSO accumulates in the ECM, causing abnormal matrix cues, and subsequent alterations of cell signalling and gene expressions, which in turn aggravate abnormal matrix composition. She assumed that this vicious circle may affect Golgi functions and have consequences on polarization, cell cycle, migration and trafficking affecting neurogenesis, neuritogenesis and synaptogenesis, especially in the cortex of affected children, leading to abnormal post-natal cortical development.

Dr. A. Fraldi closed the scientific session speaking about "Cholesterol abnormalities and lysosomal dysfunction in lysosomal storage diseases ". He explained that his research focused at investigating the mechanisms underlying lysosomal dysfunction, particularly the impairment of endocytic and autophagic pathways. He investigated the abnormalities in lysosomal membranes of LSDs and, above all, he performed his experiments on Multiple Sulfatase Deficiency (MSD), an autosomal recessive and extremely rare condition characterized by congenital loss of function of SUMF1, the enzyme required for post-translation modification of sulfatases, and Mucopolysaccharidosis type IIIA (MPS-IIIA) the most common form of MPS characterized by congenital loss of Glucosamine-N-Sulfamidase, a lysosomal enzyme required for degradation of Heparan Sulfate (GAG). Dr. Fraldi elucidated the following research main outcomes:

- Lysosomal membrane contains an abnormal amount of cholesterol-enriched regions in LSD models analyzed (MSD and MPS-IIIA)
- Key proteins which have the intrinsic capability to bind cholesterol (SNAREs, LAMP2a, others?) are abnormally retained in these membrane regions and became dysfunctional
- Critical functions of lysosomes which rely on these proteins are impaired (lysosomal fusion with other target membranes, chaperon-mediated autophagic degradation, others?)
- New therapeutic strategies may be developed on the basis of these new concepts

He then concluded his underline that autophagy failure causes accumulation of toxic substrates and dysfunctional organelles (ubiquitinated proteins, mitochondria etc.).

5th march 2010

Scientific Session: Pathophysiology and LSDs

Several talks were presented in this session; each of them gave great input for a better understanding of natural history and pathophysiology of LSDs with particular attention to the recent advances in treatment options, and in particular they emphasized the important role of an early

intervention in preventing the morbidity and mortality associated with each of the disorders. Participants gave an overview about the new recent advances on biomolecular mechanism responsible of LSDs pathophysiology with main interest in CNS neurodegeneration and mechanisms for drug delivery to the brain and related therapy possibilities.

Dr. M. Horwitz opened the session with a talk about "ER associated degradation and unfolded protein response in Gaucher disease". She briefly introduced Gaucher disease, the autosomal recessive disease characterized by accumulation of glucosylceramide mainly in cells of the reticuloendothelial system, due to mutations in the acid β-glucocerebrosidase gene. She then talked about the existing link between Gaucher and Parkinson disease focalizing her attention on Gaucher mutations in the glucocerebrosidase-encoding gene GBA1 that have been recently identified as a major cause for Parkinson disease. One of the genes associated with Parkinson disease is PARK2, encoding an E3 ligase which ubiquitinates the misfolded enzyme before its elimination in the proteasome. Based on these observations she introduced the possibility that the concurrence of GD and PD reflects an association between parkin and misfolded mutant glucocerebrosidase variants. In fact data strongly indicated that several glucocerebrosidase variants undergo parkin mediated ubiquitination and degradation in the proteasome. She also showed that mutant glucocerebrosidase variants present variable degrees of ER retention and undergo ER associated degradation (ERAD) in the proteasome.

Followed then the presentation of **Dr. J Cooper** entitled "The Neuronal Ceroid Lipofuscinoses (NCLs): a different sort of LSD?" He talked about neuronal ceroid lipofuscinoses, a group of at least ten fatal inherited storage disorders commonly known as Batten disease. Dr. Cooper took the participants through the similarities and differences among NCS and other LSDs focalizing then the attention on diseases pathogenesis. He presented data from in vivo (Human NCL and animal model of neuronal ceroid lipofuscinosis: knock out and knock in mice and large animal models) & in vitro approaches aimed to reveal insights into its pathophysiology. Acquired knowledge together with new upcoming experimental results can help to investigate pathogenesis and compare what happens in the brain in different types of the disease and possibly find an answer to the following questions: what happens where? when? And how? Does a relationship between storage material and cell death exist? Observing that not all neurons are in fact equally affected the research is now focused on understanding which types of neurons and even which parts of these neurons the disease impacts first. Moreover he discussed about the relative contribution to pathogenesis of different cells exploring in particular the neuron-glia interactions in the NCLs. In fact is still not completely known if glia play a role in NCL pathogenesis and if they can be affected by NCL as

well. All these endpoints could ultimately be important for clinical trials and development of effective treatment.

Followed **Dr. E Lloyd-Evans,** who talked about Smith-Lemli-Opitz Syndrome (SLOS), a recently identified lysosomal storage disease caused by defective cholesterol biosynthesis. He explained that it is an inherited neurodegenerative, multistorage disease due to defect of ER enzyme involved in the final step of cholesterol biosynthesis. Such alteration causes accumulation of the cholesterol precursor, 7-dehydrocholesterol (7-DHC). The similar sequence of cellular pathogenetic alterations was observed in SLOS as in NPC1 patients. The data indicate a regulatory role of sterols in maintaining correct NPC1 function but also indicate that it is sphingolipids and not sterols that cause the endocytic dysfunction seen in NPC1 disease.
He then discussed the possible role of Miglustat a glucosylceramide synthase inhibitor, as effective therapy for SLOS. He proved that it clears lysosomal storage bodies and corrects endocytic and lysosomal calcium defect. He concluded his presentation suggesting that SLOS patients may benefit from glycosphingolipid lowering drugs in combination with dietary cholesterol elevation and therefore proposed that SLOS is a 'secondary' lysosomal storage disease caused by the accumulation of a metabolite, 7-DHC, directly linked to the primary metabolic defect, ER cholesterol biosynthesis.

Scientific Session: crossing the Brain Blood Barrier and therapeutical options

In this session the discussion was about factors influencing the ability of a particular substance to cross the BBB and enter the brain, as the concentration between compartments, size of molecule (i.e. molecular weight), flexibility and conformation of molecule, amino acid composition, lipophilicity, cellular enzymatic stability, cellular sequestration, affinity for efflux mechanisms (i.e. P-glycoprotein), hydrogen bonding potential (i.e. charge), affinity for carrier mechanisms, and effects of existing pathological conditions. Moreover other peripheral factors are responsible as the systemic enzymatic stability, plasma protein binding affinity, cerebral blood flow, uptake into other tissues, clearance rate, and effects of existing pathological conditions.

The session reviewed the discovery, design and development of small- and large-molecule drugs that can efficiently cross the BBB. Development of drugs delivery to brain system includes chemistry-based methods, carrier-mediated transport (CMT) and receptor-mediated transport (RMT), use of nanoparticles technology, stem cell and viral vector. Data obtained by in vitro and in vivo assays as well as imaging methods to ascertain a drug's ability to cross the BBB and reach its target were presented.

Dr. M. Hammarlund-Udenaes talked about “Drug delivery to the CNS”. She underlined that predicting drug delivery to the brain is complicated because of the specific functions of the BBB with its active efflux and influx transporters. It is likely that there are still many undiscovered transporters at the BBB that affect currently used drugs. Also, one drug can be a substrate for several transporters. Therefore she underlined the main conceptual point that must be taken into account:

- The rate of drug entering the brain across the BBB ≠ extent of transport/distribution to the brain.
- The extent of unbound drug in the brain interstital fluid determines the effective concentration during treatment in relation to unbound plasma concentrations (Kp,uu).
- The unspecific binding in brain tissue can make the total to unbound drug concentrations in brain differ between drugs 1 – 3000-fold (Vu,brain).

In vivo investigations are therefore essential to ”map” brain distribution properties of new compounds and main rapid procedures were described. Related outcomes indicate that:

- Unbound extent ratio (Kp,uu) is to be preferred to relate brain delivery to pharmacological action.
- Uptake and efflux with several transporters likely more common than single transporter effects on drugs.
- Phys-chem properties to be optimized in drug discovery are quite different when taking unbound drug transport into consideration (less lipophilic compounds).
- CSF is an o.k. measure of unbound drug concentrations in brain.

The session was then closed by **Dr. R. Katona** who gave a talk about “A potential strategy for the treatment of neurological disorders: combined mammalian artificial chromosome-stem cell therapy.” He underlined that mammalian artificial chromosomes (MACs) are safe, stable, non-integrating genetic vectors with almost unlimited therapeutic transgene-carrying capacity. He described how Artificial Chromosome Element (ACE) was delivered into mouse stem cells and pluripotent mouse embryonic stem cell lines were established. ACE technology (applied combination of ACE and stem cell technologies) offers a new strategy for stem cell-based therapy, the efficacy of which was confirmed and validated by using a mouse model of a devastating monogenic disease, galactocerebrosidase deficiency (Krabbe’s disease). He presented data showing that transgenic mouse embryonic stem cells could be therapeutically used to create chimeric mouse whose lifespan was twofold increased. He concluded his presentation observing that although the model is still under development and more investigation are required, the development of this strategy for delivering therapeutic genes in stem cells represents an important step for treating genetic diseases and producing cell types for cell replacement therapies.

After lunch followed the presentation of **Dr. J. Kreuter** who gave a talk entitled “On the mechanism of nanoparticle drug deliver across the Blood Brain Barrier: transport kinetics and influence of targeting ligand attachment”. He firstly showed some graphs representing Doxorubicin bound to different kinds of nanoparticles and in particular he underlined that the kinetics of the doxorubicin transport across the BBB evaluated in rats by the capillary depletion was significantly improved when drug were transported into the brain by the polysorbate 80-coated PBCA nanoparticles. He then discussed about the “Mechanism of Nanoparticle Transport Across the BBB Using Nanoparticles” (nanoparticles acts as Trojan Horses) and showed that transmission electron microscopy indicates that the mechanism appears to be receptor-mediated endocytosis followed by transcytosis and in particular he stressed this conclusion:

- The mechanism of drug transport with nanoparticles is receptor-mediated endocytosis followed by transcytosis.
- This transport will occur if there is a respective receptor on the brain capillary endothelial cells for the targeting ligand that is present on the surface of the nanoparticles.
- The targeting ligand can be bound covalently or by adsorption.

Dr. V. Gieselmann spoke about “Transport of Arylsulfatase A across the BBB“. He at first gave a brief introduction of MLD explaining that it is an inherited autosomal recessive disease commonly caused by a mutation in a gene called arylsulfatase A (ASA). He explained that the gene defect induces the accumulation of sulfatides in the myelin. The storage provokes myelin structure disruption causing demyelination in both the central nervous system and in the peripheral nervous system. He then presented some data about Sulfatide storage in brain of ASA deficient mice after ERT showing a surprisingly reduction of sulfatide accumulation in the brain indicating that a small amount of the enzyme was able to pass the BBB. These results were confirmed in an in vitro model of the BBB (primary porcine brain capillary endothelial cells) and the following conclusion was established:

- long term high dose enzyme replacement leads to a dose dependent sulfatide reduction in the central nervous system
- Intravenously injected Arylsulfatase A is detectable in parenchymal brain homogenate
- Transport of ASA across the (in vitro) BBB is largely charge dependent
- Cationization of ASA may improve therapeutic efficacy
- M6P- dependent and adsorption mediated transcytosis contribute to BBB transport

Therefore cationization and an increased content of mannsoe-6-phosphate may increase the delivery of enzyme across the BBB in vivo.

He highlighted the fact that the BBB represents a formidable obstacle for drug delivery to the brain. He then reviewed new different strategies and approaches allowing the improvement of the drug delivery to brain targets. He showed how developed delivery techniques could include an invasive drug delivery (intra cerebroventricular, intra theca, intra cerebral) or non invasive drug delivery (trough passive diffusion, carrier mediated transport, receptor mediated transcytosis, adsorptive mediated transcytosis). Many of these approaches, though showing potential efficacy continue to have limits for clinical applications. The main issue is to find a balance between safety/efficacy, between brain specificity/brain toxicity (Polyamines and CPPs as Trojan horses vector for AMT), brain specificity/efficiency (peptides as Trojan horses for RMT) and last between specificity/ efficiency-Toxicity (nanotechnology). This statement stressed the need to improve the understanding of basic mechanisms of AMT and RMT at BBB.

Followed **Dr. A. Zimran** who gave talk entitled "OTC PC: from rats and type I to type III Gaucher disease: give Ambroxol a chance!". He briefly introduced Gaucher disease features and showed data of patients who underwent ERT showing a dramatic improvement in general symptoms, but underlining that unfortunately ERT is unlikely to impact cognitive/intellectual features of neuronopathic Gaucher disease. He illustrated results from a clinical trial in GD3 using miglustat (Zavesca; Actelion Pharmaceuticals) that failed because of inability to meet end-points. Also the use of the pharmacological chaperone (PC) isofagomine (Plicera, AmicusTherapeutics Inc) which is potentially able to cross the BBB, failed phase 2. He then presented data from the Pilot project of Ambroxol (ABX) for untreated patients with type I Gaucher disease at Shaare Zedek Medical Center. ABX is a mucolytic agent commonly indicated for many respiratory conditions easily available over-the-counter in many countries. The trial demonstrated that patients who had consistent positive results were the thinnest patients, suggesting a need for higher doses of ABX as PC for Gaucher disease. Data encourage carrying on the research focalizing attention on the following points:

- A more robust proof of concept is needed for ABX in type I Gaucher disease
- Proof of concept of ABX capability in traversing the BBB
- Phase II trial of ABX in patients with type 3 Gaucher disease (2 years, placebo-controlled?)

Dr. B. Bigger discussed then about the role of genistein in the treatment of MPSIII, a group of very similar diseases, affecting children in the first decade of life, caused by lysosomal enzyme deficiencies in catabolism of heparan sulphate (HS). It causes progressive cognitive and later motor decline, behavioural and sleep problems, hyperactivity and ultimately death in their mid teens. He then focalized attention on the different treatment approaches for MPSand in particular he discussed about Genistein, the tyrosine kinase inhibitor that can be purified from soya beans as a

food supplement or synthesised in its pure aglycone form. It blocks GAG production in patient cells in culture from all MPS types tested so far. He showed results from a genistein aglycone dose finding study for reduction of liver and brain GAGs in MPSIIIB mice with existing storage. He reported that

- genistein aglycone effectively reduces pathological heparan sulphate and lysosomal size in the brains of MPS IIIB mice by a third.
- Neuroinflammation was reduced by about a sixth and secondary pathology in the brain may be improved; peripheral storage is reduced.
- Behavioural abnormalities corrected in 8 month old MPS IIIB mice.
- No toxic side effects were observed.
- Genistein will not cure MPS IIIB - but appears to delay disease progression – at least in mice.
- Doses used to see these effects (160 mg/kg/day) are not achievable with the supplement form of genistein - Equivalent doses of Soyfem may not be as effective.
- Significant phase I human safety data already exists for lower doses of genistein aglycone.

He then concluded his presentation putting in evidence that a clinical trial is essential to determine efficacy/safety of genistein aglycone in patients.

Followed then **Dr. S. Waddington** who spoke about “perinatal gene therapy for lethal genetic diseases” Ho started his presentation showing some scenes from famous movies about rare genetic diseases. He underlined that several inherited neurological diseases are characterised by neurodegenerative changes at or around birth. Prognosis remains dismal and for several of these diseases, including acute neuronopathic Gaucher Disease, there is no treatment and palliative care remains the only option.

He then explained the reason why Fetal or neonatal gene therapy may provide a means of pre-empting the rapid onset of neurological damage in these diseases.

- Immunotolerance: study of fetal injection compare to adult mice injections shows that fetal administration results in immunotolerance
- Efficiency
- Early cure (possibility to prevent the advent of an early disease, mice are therefore injected in the uterus)

Recently, AAV9 has been shown by others to transduce the brain and spinal cord of mice, cats and macaques following intravenous injection. Considering that mouse model replicates human disease a comparison of gene expression after intravenous injection into fetal and neonatal mice was undergone. Results clearly show that AAV9 may serve as a useful tool for dissection of the role of different cells and tissues underlying the pathogenesis of disease models of neurodegenerative disease. Furthermore they may provide a pathway towards therapeutic intervention.

Dr. M. Vanier concluded the session giving a very enjoyable plenary lecture entitled "Niemann-Pick C disease: the enigma and the challenges". She briefly introduced Niemann-Pick C disease (NP-C) as a severe autosomal recessive disease with neurovisceral manifestations which is not so rare among rare diseases. It is due to defects on two different genes NPC1 and NPC2 having similar clinical and biochemical phenotype as proved in patients and mouse models. She underlined that NPC is not just a simple block in degradation of one specific lipid but a major trafficking problem. According to data presented it's clear that, among lysosomal neurolipidoses, Niemann-Pick C disease (NP-C) is atypical in many ways. She then presented data from animal study aimed at investigating the role of the two late endosomal/lysosomal proteins (transmembrane NPC1 or soluble NPC2). It was shown that NPC1 and NPC2 function in a no redundant coordinate fashion. In extra neural tissues and cells, the most obvious metabolic lesion involves a unique impairment in processing and utilization of endocytosed cholesterol. This alone might explain the complex lipid storage (not truly dominating lipid storage, and with a particular characterization in the brain where neurons accumulate mostly GM2 and GM3 gangliosides, and much less cholesterol) observed in patients and animal models. More data must be acquired and deeper investigation should be done in order to better understand the diseases pathophysiology and propose approaches to therapy. Miglustat is currently the only approved disease-modifying drug while encouraging studies with ß-cyclodextrin derivate show an important experimental therapeutic effect although mechanisms of action are still not clear.

6th march 2010

Scientific Session: B4B and Europe

In this session speakers emphasized the fact that within the EU paediatric neurodegenerative disorders are considered "Rare" and that because of the low-prevalence of these disorders, there is often a striking lack of information, research, treatment and expert availability. There is also often a significant delay before a definitive diagnosis is achieved. Therefore, as underlined by **Dr. M. Scarpa** the 2010-2012 main goal is to make B4B visible. He explained how the B4B Foundation is progressing and he further updated audience about what the B4B Foundation has done and is doing presenting a brief slides excursion showing all the already achieved targets and the important meetings and workshops organized by B4B aimed at:

- stimulating interest on rare diseases as models for the therapy of adult and more common brain diseases,
- alerting the Political Members on the needs of the families and the Scientific Community to continue to devote funds for research and social assistance and

- starting collaboration with the EU Commission for the discussion of themes of interest for our community.

At this point he presented then the renewed Brains for Brain Web site: www.brains4brain.org and he explained that it represents an amazing interactive tool for increasing B4B visibility and awareness.

There was an active and productive discussion regarding European funding opportunities and necessity of a collaborative research effort to focus the available multidisciplinary European brain-power with relevance to tackling pediatric neurodegenerative diseases. The necessity to create a solid international group focused on the development of an effective therapy for treating LSDs and other important neurological diseases such as Alzheimer's, Parkinson's Disease, and epilepsy was underlined, It was then highlighted that the multidisciplinary group, together with family association must work as a network in order to acquire political support and stimulate Scientific Community attention on paediatric neurodegenerative diseases aimed to obtain a call for a neurodegenerative specific research grant.

Unfortunately **Prof. Olga Golubnitschaja** couldn't attend the meeting and **Dr. M. Scarpa** presented her slides about "Asphyxiated newborns - common origin but individual outcomes: Time for new guidelines in personalised healthcare**"** He focused the attention on the fact that research has been focalized in producing therapies for disorders but now we have to think about a new concept: Prediction for Prevention = Medicine of Future. He stressed that we are moving toward a personalized medicine and it means to give the right therapy at the right moment in the right way. Unfortunately regarding rare genetic diseases there are, so far, there are very few biomarkers telling us how the diseases is progressing and how to customize the therapy. He then introduced EPMA-Networking: an Umbrella European Association covering over 35 countries whose objectives (similarly to the B4B Foundation's one) are:

- Raising awareness and recognition of Predictive, Preventive and Personalised Medicine (PPPM) throughout all Member-countries of the European Union and Associated countries,
- Global networking of leading experts in PPPM, Multidisciplinary consortium with complementary expertise in PPP-related fields,
- Innovative PPPM-technologies,
- International validation of new biomarkers and therapeutic targets,
- Translation between research & industry,
- Standardisation,
- Creation of PPPM-clinics clustered together,
- Cooperation with patient organisations,
- Education of new generation of professionals,

- Development of PPPM-related strategies in healthcare,
- Public Relation

He then went through EPMA board, EPMA strategic partners, The EPMA-Journal and book series,…and particularly he announced the next opening of the IMPLEMENTATION PPPM-Centre in Brussels (Open 06-2011), a location where projects can be performed through multitasking technology building. He also communicated that the EPMA first meeting will be organize in Bonn and invited people to participate.

Dr. M Scarpa also anticipated the incoming research funding opportunities for the Health Theme of FP7 illustrating in particular the one of interest for B4B and highlighted that B4B is an organization which is entitled to be recognized as the meeting point of all the stakeholders interested in neurodegenerative rare diseases and moreover B4B is becoming to be recognized as a valuable tool on the worldwide scene of science and health care. He underlined that the collaboration with important EU organizations have been initiated and now there is the necessity to form a core group of people (4-5) who will help in constructing a political visibility and awareness on the activity of B4B.

Dr. L. Kristiansen gave the last talk of the session entitled “Introduction to the ESF Forward Look on personalized medicine.” He explained that the European Science Foundation (ESF; www.esf.org) is an independent non-governmental organization of 78 member Organizations (research funding organizations, research performing organizations and academies and learned societies) in 30 countries. He underlined that the ESF provides a common platform for its Member Organizations acting as a catalyst for the development of science by bringing together leading scientists and research funding agencies to debate, plan and implement pan-European initiatives and to explore new directions for research at the European level covering all scientific domains. He then anticipated that ESF is in discussion with EUROHORCS about a possible merger of the two organizations to create a united voice for science in EU giving rise to a powerful driving force. Through its activities, the ESF serves the needs of the European research community in a global context. Its mandate is underpinned by the values of excellence, openness, responsiveness, pan-European, ethical awareness and human values. Regarding B4B and ESF joint programme he underlined the events that took recently place:

- exploratory workshop, Frankfurt (March 2010)
- TC Jan. 2011 about follow-up activities (M. Scarpa)
- Identification of potential mutual synergy areas –possibly shared interest around the ESF Forward Look on Personalized Medicine

He concluded his presentation communicating the establishment of ESF Forward Look on personalized medicine for the European Citizen and describing the promise of the personalized medicine putting in evidence the rapid development, technology-driven, affecting basic constrains and dynamics of society, focus on health and disease specifying that healthcare is on the brink of a revolution precipitated by dramatic advances in biomedical research. The ability to distinguish, at the molecular level, what makes one person different from another lies at the heart of this fundamental shift. Combined, these developments will change our approach to medicine from finding cures towards individualized prediction, diagnosis, treatment and prevention.

Scientific Session: B4B and biotech collaborations

In this last session the role of the industries in driving innovation for new therapeutical approaches for true unmet needs was discussed.
Pharmaceutical Companies presented their research studies aimed to develop new therapies to treat central nervous diseases. Existing therapies for several LSDs (i.e. MPS sub-classes) have been approved and have demonstrated clinical systemic benefits. However, a significant need exists for additional treatment options as the present therapies do not fully address the needs of clinicians and patients. Patients continue to experience debilitating and sometimes fatal symptoms. The main common goal is to produce medicines effective not only in isolated cells grown in tissue culture but also able to cross the blood-brain barrier in a living animal or person. Factors which control the entry into the brain of medicines and other therapeutic agents which may be helpful in treating central nervous disease were so reviewed and discussed in this last scientific session. By understanding the factors which allow some substances to cross the blood-brain barrier and others not to, the design of new more effective medicines for the treatment of central nervous disease becomes possible. Altogether the speakers communication emphasized the concept that the development of drug formulations suitable for direct delivery to the CNS is ongoing and preclinical studies are planned and partially already running bringing new hope to patients suffering from neuronophatic LSDs.

The session was started by **Dr. P. Calias** from Shire who discussed about “Intrathecal (IT) delivery of recombinant lysosomal enzymes”. He explained that one of the major aim is to design, develop and validate efficient strategies to deliver effective therapy that reverses LSD neurodegeneration to the CNS. This Protein therapeutic delivery is an extremely difficult challenges and is an underdeveloped area of research. He then provided data on Shire most recent advances in intrathecal (IT) delivery of enzyme replacement therapies for CNS indications. Considering the great debate between intrathecal (IT) or intracerebroventricular (ICV) administration he presented

some head to head results of comparison in Cynomolgus monkey (RhAsait/ICV) showing that IT was normally well tolerated and rapidly exported with a deposition gradient from the cerebral cortex to the ventricular white matter. He also presented data obtained using Iodinateidurinate 2 sulfatase showing that the distribution is the same and therefore he concluded his presentation asserting that IT administration affectively delivers enzymes to the appropriate brain tissue sites as well as to the affected organelles. Is still not clear how the enzyme is transported.

Followed **Dr. R. Gabathuler** from BiOasis Technologies who gave a talk entitled "improved pharmacological chaperones for the treatment of neuronopathic Gaucher and Parkinson's disease". He explained that BiOasis is a biopharmaceutical company actually working on diagnosis and treatment of rare disease and in particular works on a physiologic route to deliver drugs on the brain. He introduced a newly developed peptide vector for drug delivery into the brain, called P97. The uptake is mediated by a receptor which has been related to the family of LDL Receptor related Protein (LRP). They use glutathione transporter targeted by G technology of ToBBB technology BV (liposome coated) and they look at the distribution of the protein. Results from recent studies show that p97 is very rapidly transported across the BBB into the brain parenchyma thanks to the involvement of a receptor of the family of LDL receptor protein.
Analysis of P97-cy5.5 uptake in HBEC shows that P97 receptor is localized in brain parenchyma while the use of P97 as a carrier of drugs shows that P97 can be applied to treat LSD brain diseases as P97 has the potential to address most of LSDs shuttling a variety of compounds ranging from small anti-cancer agent to larger biologics such as enzymes.

Dr. S. Clark from Amicus Therapeutics "Improved pharmacological chaperones for the treatment of neuronopathic Gaucher and Parkinson's disease. He at first explained what the chaperon terminology refers to underlining that pharmacological chaperones are compounds that selectively bind and stabilize mutant & wild-type proteins, to facilitate folding and restore function. He underlined that pharmacological chaperones are designed to be selective, potent, and reversible and then showed data obtained using the pharmacological chaperone AT2101 that was able to increase GCase levels in healthy volunteers. He then highlighted that *Gba* mutations represents a significant risk factor for development of Parkinson's disease, therefore they used Gaucher and PD mouse models to investigate the role of key chaperone molecules. Mice with reduced GCase activity but normal murine synuclein accumulated glucosylceramide (GlcCer) in the brain and Mice with reduced GCase activity accumulated brain α-synuclein. Moreover Mice with reduced GCase activity accumulated brain α-synuclein and in particular it was noticed that some synuclein accumulation appeared to be within microglia. The following hypothesis was then formulated: If Gaucher carriers are at risk for PD as a result of decreased GCase activity, which

leads to synuclein accumulation, then Pharmacological Chaperones that increase GCase activity should decrease synuclein accumulation. Experimental data from Thy-1-h*SNCA* mice model confirmed such hypothesis showing that AT2101 enhance GCase activity in the CNS, reduces α-synuclein accumulation, prevent accumulation of α-synuclein/improve synuclein-dependent behaviors, reduce spleen GlcCer. He then concluded his presentation specifying that pre-clinical efficacy and IND-enabling studies with AT3375 are ongoing, supported by a grant from the Michael J. Fox Foundation.

The session was then closed by **Dr. T. Kirkegaard,** from Danish Cancer Society, gave a talk entitled "HSP70 stabilizes lysosomes and reverts Niemann-Pick disease-associated pathology".
Heat shock protein 70 (HSP70) is an evolutionarily highly conserved molecular chaperone that promotes the survival of stressed cells by inhibiting lysosomal membrane permeabilization. He explained that Hsp70 stabilizes lysosomes by binding to endolysosomal anionic phospholipid bis(monoacylglycero)phosphate (BMP), an essential co-factor for lysosomal sphingomyelin metabolism. Experiments on a working model of HSP70 showed that in acidic environments Hsp70 binding stimulates acid sphingomyelinase (ASM) activity that in turn stabilizes lysosomes therapeutic potential and reverts lysosomal pathology. Data from experiments with RhHSP70 in cells from patients with Niemann–Pick disease (NPD) A and B (typically characterized by an associated marked decrease in lysosomal stability) showed that HSP70 rescues lysosomal pathology (complete reversion of lysosomal pathology). He then concluded his presentation claiming the importance of such results in offering exciting possibilities for the development of new treatments.

Dr. M. Scarpa and **Dr. D. Begley** closed the workshop highlighting how this workshop confirmed many of the positive impressions we have had for the previous B4B workshops and that it was a very fruitful one and definitely a success. Thanking all participants for their hard work of workshop and for their outstanding contributions (including invited speakers) they underlined that the workshop objectives of sharing knowledge and experience and initiating joint work were achieved. Maurizio and David pointed out that the workshop saw a number of emerging themes and identified some future actions and that it was interesting to note the extent of common concerns among participants across the range of different kinds of projects, organizations, subject and data or content types that were represented. They appreciated that one of the most interesting things to emerge from the workshop was the fact that a number of contacts and ideas for future collaborations were established.

I apology if something is missing or not fully reported in this document.

SCIENTIFIC REPORT

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