

---

## *Research Presented*

**2004 Lysosomal Diseases and the Brain Conference**

**Organized by the Children's Gaucher Research Fund**

### *--- Speaker Abstracts ---*

---

#### **The Blood-Brain Barrier: Delivery of Therapeutics to the CNS, the Problems and the Possibilities.**

David Begley Ph.D. - [david.begley@kcl.ac.uk](mailto:david.begley@kcl.ac.uk)

*Senior Lecturer in Physiology, Centre for Neuroscience Research, Hodgkin Building, Kings College London – Guy's Campus, London SE1 1UL, UK*

All organisms with a complex central nervous system require a blood-brain barrier (BBB). The fundamental functions of the barrier are two-fold.

Firstly, it enables the creation of a separate and extremely stable intracerebral extracellular fluid compartment consisting of the cerebrospinal fluid and the brain interstitial fluid. Within these protected compartments the composition of the extracellular fluid can be precisely regulated in terms of solute concentrations. This stability is essential as the CNS relies on accurate synaptic transmission, inhibition and summation, in order to perform its complex integrative functions. Unless the synapse can operate against this very stable background accurate synaptic transmission becomes impossible. The somatic extracellular fluid contains many neurotransmitters and other neuroactive substances whose concentrations vary widely within short periods of time. The CNS could not tolerate these significant variations in composition of the general extracellular fluid that occur on a regular basis.

Secondly, the BBB has a neuroprotective function. In a highly complex tissue such as the CNS, where cell division is either absent or a rare event, any acceleration in cell death and neuronal attrition will cause premature degenerative disease and pathology. Many potentially neurotoxic substances are being continuously ingested in the diet, or are generated by metabolism. The BBB is therefore crucial in

limiting the access of these potentially damaging xenobiotics and metabolites to the CNS by either blocking their entry or actively removing them from brain.

In mammals the BBB is formed by the cerebral capillary endothelial cells and the blood-cerebrospinal fluid barrier (BCSFB) by the epithelium of the choroid plexus. The physical barrier to solute diffusion is formed by transmembrane proteins which create tight junctions between either the cerebral endothelial cells or the epithelial cells of the choroid plexus. These tight junctional complexes effectively abolish any paracellular diffusional pathways between the cells. The creation of the BBB and the BCSFB therefore effectively produces a seal for the CNS against blood-borne polar solutes. Therefore, in order to receive a sufficient supply of essential polar metabolites and nutrients, such as glucose and amino acids, the cerebral capillary endothelium must contain specific transport mechanisms to deliver these solutes in sufficient quantity across the barrier. Solutes which are more lipophilic are able to diffuse directly across the barrier at a rate which is increased in proportion with an increased lipophilicity. A significant number of lipophilic substances do not however penetrate into the CNS as rapidly as might be expected. These lipophilic low permeability substances are often substrates for specific and active efflux transporters, the ATP-Binding Cassette (ABC transporters), which can actively eject them from the brain. Many of these lipid-soluble substrates may be potentially neurotoxic xenobiotics or metabolites and often they may be systemically administered therapeutic drugs which then become effectively excluded from the brain. For larger solutes such as peptides and proteins specific transport mechanisms into the brain also exist. On electron-microscopical examination there is, however, apparently little obvious vesicular traffic across the CNS vascular endothelium. Never-the-less it is clear that a transcytotic mechanism of transport for many macromolecules is highly significant. The BBB and the BCSFB also contain significant enzyme activities which can hydrolyse and conjugate solutes. Thus the brain barriers, in addition to being physical and transport barriers are also a metabolic barrier and shield for the brain. Many of the products of these “barrier” enzymes are then substrates for the efflux BBB ABC transport mechanisms.

These various characteristics of the blood-brain barriers make them a formidable obstacle when attempting to deliver therapeutics to the brain. A number of strategies are available to enhance drug entry and to target drugs to the brain. These involve informed drug design so that the physico-chemical properties of a drug can be optimised for maximal penetration and CNS effectiveness, for example, by either enhancing passive penetration or a pro-drug chemical-delivery system strategy. Alternatively the BBB can be by-passed altogether by direct

injection or infusion into the brain or its cavities, or by employing novel routes of entry such as the olfactory epithelium. The tight junctions in the cerebral endothelium can also be manipulated by techniques to modulate the paracellular pathway to allow drug entry. Drugs may also be designed as false substrates which are then able to utilise the endogenous transporters present in the BBB or therapeutics may be incorporated into complex vector constructs employing monoclonal antibodies or other targetors to gain entry. In addition, cell penetrating peptide systems, or particulates such as nanoparticles and liposomes can be designed which are able to carry therapeutics into the CNS. An attractive feature of particulate systems is that they can potentially carry relatively large amounts of high molecular weight substances into the CNS. Also, knowledge of the mechanisms by which ABC transporters operate opens the possibility of designing potent specific inhibitors for these efflux pumps and also introduces the concept of designing reactivity with the ABC transporters out of chosen drugs by appropriate molecular modifications.

The functions of the normal blood-brain barriers will be discussed and the methods for optimising CNS drug delivery will be reviewed.

### **References:**

*Drug Delivery and Targeting to the Brain.* (2002) J. Drug Targeting, **10** (4). (Special issue). Eds. DJ Begley and J Kreuter.

Begley DJ and Brightman MW (2003) Structural and functional aspects of the blood-brain barrier. *Progress in Drug Research*, **61**. Eds. L. Prokai and K. Prokai Tatrai. Birkhauser Verlag, Basel, Switzerland.

Begley, DJ (2003) Efflux mechanisms in the CNS: A powerful influence on drug distribution within the brain. In: *Blood-Spinal Cord and Brain Barriers in Health and Disease*. Eds. HS Sharma and J Westman. Elsevier/Academic Press. San Diego, USA.

Begley DJ (2003) Understanding and circumventing the blood-brain barrier. *Acta Paediatrica Scand. Suppl.*, **443**, 83-91.

Begley DJ (2004) ABC Transporters and the blood-brain barrier. *Current Pharmaceutical Design*. (In press)

---

## **Active-site specific chaperone therapy: Implications in lysosomal storage disorders.**

Jian-Qiang Fan, Ph.D. - [jfan@amicustherapeutics.com](mailto:jfan@amicustherapeutics.com);

*Vice President of Research, Amicus Therapeutics, Inc., North Brunswick, New Jersey*

Small molecules are proposed as potential drugs for the treatment of lysosomal storage disorders (LSDs), which are caused by deficiencies in lysosomal enzymes. Certain mutations in these disease-causing enzymes result in the synthesis of improperly folded proteins that are retarded in the endoplasmic reticulum (ER) and degraded by ER-associated degradation, although these proteins might be enzymologically active if they could be properly transported to the lysosomes. At sub-inhibitory concentrations, potent competitive inhibitors of the mutant enzymes can act as active-site specific chaperones (ASSCs) to induce or stabilize the proper conformation of the mutant enzyme. This promotes normal trafficking through the ER secretory pathway, ultimately increasing the residual enzyme activity in lysosomes. Once a mutant enzyme:inhibitor complex is in the lysosomes, the inhibitor can then be replaced by highly concentrated (accumulated) substrate because the dynamic exchange of competitive inhibitor and substrate is dependent upon the relative concentrations of each.

A significant number of patients with point mutations, or small in-frame deletions, present detectable residual enzyme activity in Fabry disease and Gaucher disease. An increase in a fraction of the residual enzyme activity in the LSDs is expected to result in substantial clinical benefit for patients. Cultivation of Fabry or Gaucher cells with ASSCs results in a substantial increase in residual enzyme activity, which could have a therapeutic impact on the disease development in these patients. The biochemical efficacy of an ASSC for Fabry disease was shown in a transgenic animal model. Small molecule drugs should pass more readily through the blood-brain barrier and, thus, have the potential to treat LSD patients with CNS involvement. Since these small molecules are likely to be orally active, they should have numerous advantages including easy administration, greater affordability and convenience, provided they are non-toxic and demonstrate long-term safety. This therapeutic strategy of using functional chemicals as ASSCs could be broadly applied to other LSDs that are caused by misfolding of mutant proteins.

---

## **Pathophysiology of type 2/3 Gaucher disease**

Tony Futerman Ph.D. - [tony.futerman@weizmann.ac.il](mailto:tony.futerman@weizmann.ac.il)

*Professor, Department of Biological Chemistry, Weizmann Institute of Science, Israel*

I will discuss our recent work examining the interplay between defective calcium homeostasis and neuronal dysfunction in type 2 and 3 Gaucher disease. These studies include analysis in animal models of the role of glucosylceramide (the lipid that accumulates in Gaucher disease) on calcium mobilization via the ryanodine receptor, and studies in human brain Gaucher tissue showing a correlation between levels of glucosylceramide accumulation, calcium release and clinical severity. In addition, I will discuss other metabolic changes in Gaucher brains, including recent data from DNA arrays, and metabolic changes in other models of sphingolipid storage diseases that together may provide an integrated picture of the underlying biochemical mechanisms responsible for neuronal and brain dysfunction in this class of diseases.

---

## **Animal models and pathogenesis of Gaucher disease**

Greg Grabowski M.D. - [greg.grabowski@cchmc.org](mailto:greg.grabowski@cchmc.org)

*Director, Division and Program in Human Genetics, Children's Hospital Research Foundation of Cincinnati, Cincinnati, Ohio*

A significant inhibition to the understanding of Gaucher disease has been the lack of useful mouse models. Previously, efforts to develop such models led to lethality shortly after birth or the lack of significant biochemical/histological involvement of the major organs in Gaucher disease. We have developed viable mouse models of non-neuronopathic and neuronopathic Gaucher disease variants that exhibit both biochemical and histological findings similar to the human disease. A total of 7 variant genotypes show variation in the degree of pathologic involvement from very little to quite significant involvement of the liver, spleen and lungs. The N370S/N370S genotype is inconsistent with survival beyond 24 hr. However,

variants with V394L, D409H and D409V do survive for periods of over 1 year without evidence of CNS involvement. The progressive biochemical, histological and transcriptome involvement will be presented in these mice. In addition, models based on the V394L or D409H Gaucher disease genotypes and the prosaposin knockout have CNS involvement and extensive tissue infiltration by Gaucher cells including liver, spleen, lung and bone marrow. Similar histological and genetic studies of the progressive pathology will be discussed in detail. These models provide a resource for pathobiological and functional genomic assessments of Gaucher disease as well as potential therapeutic assessment tools prior to human studies.”

---

### **The use of human central nervous system stem cells (hcns-sc) to treat Infantile Neuronal Ceroid Lipofucinosiis.**

Pedro Huertas, M.D. Ph.D. - [pedro.huertas@stemcellsinc.com](mailto:pedro.huertas@stemcellsinc.com)

*Acting Chief Medical Officer - StemCells, Inc., Palo Alto, California*

Neural stem cell transplantation has the therapeutic potential for treatment of neurodegenerative diseases or CNS injuries. Prospective isolation of human central nervous system stem cells (hCNS-SC) yields a highly purified neural stem cell population that can extensively expand in vitro to generate cell banks. Using fluorescent-activated cell sorting we have isolated a population of CD133<sup>+</sup> CD24<sup>-/lo</sup> cells that reproducibly expand in defined serum-free conditions as neurospheres. The in vivo biological potential of the expanded hCNS-SC were tested by transplantation studies into immunodeficient NOD-Scid mouse brains. Approximately 50 different hCNS-SC derived neurosphere cell lines have been transplanted into the brains of neonatal and adult NOD-Scid mice (n>2000). Over 1000 individual mouse brains (6-50 wk post-transplantation), have been evaluated for engraftment, migration and differentiation of human cells. Engraftment was found to be durable and reproducible showing extensive migration throughout the brain including the olfactory bulb, cerebral cortex, basal ganglia, hippocampus, pons/medulla and cerebellum. The progeny of hCNS-SC differentiated into neurons, astrocytes and oligodendrocytes in a site-specific manner with no evidence of tumor formation being observed.

Recently, we have shown the efficacy of hCNS-SC transplantation in several preclinical models including spinal cord injury, myelin deficiency, and a lysosomal

storage disorder (LSD). Because hCNS-SC neurosphere cells can engraft and migrate widely, these cells could potentially be used to correct LSDs by providing the missing enzyme. We have chosen to test this hypothesis in a mouse model of infantile Neuronal Ceroid Lipofuscinosis (INCL), using the palmitoyl-protein thioesterase 1 (PPT1) knockout mouse. INCL is fatal neurodegenerative disease, caused by mutations in the PPT1 gene. We tested hCNS-SC for PPT1 enzyme production and secretion. hCNS-SC neurosphere cells produce high levels of PPT1 as well as tripeptidyl peptidase (TPP1), the missing enzyme for late infantile NCL patients. In vitro, both PPT1 and TPP1 enzymes are secreted from hCNS-SC and accumulated in the culture media. When PPT1<sup>-/-</sup> fibroblasts were co-cultured with hCNS-SC by transwell system, the mutant fibroblasts displayed the uptake of PPT1 enzyme. Thus, hCNS-SC can deliver PPT1 or TPP1 enzyme upon transplantation. Transplantation of hCNS-SC into neonatal PPT1<sup>-/-</sup> mice results in robust engraftment and wide distribution of cells in the cortex, hippocampus and cerebellum. Since the INCL disease in human and mouse is associated with accumulation of autofluorescent storage material and leads to neuronal cell loss such as the Purkinje cells, we tested these parameters to demonstrate efficacy. Expression of human PPT1 enzyme can be detected for >6 months post transplant. Transplanted brains show a reduction in autofluorescent storage material (p<0.05), and reduction in the loss of Purkinje cells (p<0.05). We are currently testing whether cell therapy can be used to treat other lysosomal storage diseases. .

---

## **New Horizons for SRT in Glycosphingolipid Storage Diseases**

Olivier Morand, Ph.D. - [olivier.morand@actelion.com](mailto:olivier.morand@actelion.com)

*Life Cycle Director, Business and Science Affairs, Actelion Pharmaceuticals Ltd, Allschwil, Switzerland*

Zavesca® (miglustat) capsule is the first oral drug in a new class known as substrate reduction therapy (SRT), that is FDA approved for the treatment of type 1 Gaucher patients for whom enzyme replacement therapy is not a therapeutic option (Pastores and Barnett, 2003). In Gaucher disease, glucocerebrosidase, the enzyme responsible for the catabolism of the substrate glucosylceramide, is deficient, causing the toxic accumulation of the glycosphingolipid (GSL) substrate in tissues, primarily macrophages, which may lead to various clinical features including hepatosplenomegaly, fatigue, anemia, thrombocytopenia and skeletal complications. The goal of treatment with SRT is to restore metabolic balance

between the production and the degradation of the substrate, by partially inhibiting its synthesis, and thereby relieving the deficient glucocerebrosidase enzyme of excessive substrate burden. Three registration studies have been conducted with Zavesca® in type 1 Gaucher patients leading to its approval in the Europe Union, the United States and Israel. In these studies, Zavesca® has been proven safe and effective in reducing liver and spleen enlargement, and increasing the hemoglobin and platelet counts in patients. Reduced bone complications were also observed in some patients.

Similarly, miglustat would have the potential to reduce the accumulation of other glucosylceramide-derived GSLs caused by inborn errors of metabolism with partial loss of activity of other lysosomal hydrolases such as  $\beta$ -hexosaminidase A in Tay-Sachs disease or  $\beta$ -hexosaminidase B in Sandhoff disease. Using two different inhibitors, miglustat and its epimer NB-DGJ, Platt et al (2003) have shown that inhibition of glucosylceramide synthase was effective in reducing the accumulation of GSLs in the CNS and visceral tissues, delaying the onset of neuromotor symptoms, the inflammatory process and disease pathogenesis, and increasing life expectancy in mouse models of Tay-Sachs and Sandhoff disease. In addition, miglustat was tested in murine and feline models of Niemann-Pick C, a severe genetic neurological disease associated with a defect in intracellular lipid trafficking. Niemann-Pick type C animals treated with miglustat showed delayed onset of neurological dysfunction, increased life expectancy (in mice), and reduced GSL accumulation and accompanying neuropathological changes (Zervas et al, 2001). Altogether the data collected in those animal models indicate that miglustat achieved therapeutic levels in the CNS.

On this basis, three clinical trials have been initiated in type 3 Gaucher disease, Late Onset Tay-Sachs and Niemann-Pick type C patients, which will be discussed. These one-year, open-label, controlled studies will determine the efficacy and the tolerability of miglustat in the treatment of these rare neurological lysosomal storage diseases with high unmet medical needs.

## **References**

Pastores GM and Barnett NL (2003) Substrate reduction therapy: miglustat as a remedy for symptomatic patients with Gaucher disease type 1. *Expert Opin. Investig. Drugs* 12, 273-281.

Platt FM, Jeyakumar M, Andersson U, Heare T, Dwek RA and Butters TD (2003) Substrate reduction therapy in mouse models of the glycosphingolipidoses. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 947-954.

Zervas M, Somers KL, Thrall MA and Walkley SU (2001) Critical role for glycosphingolipids in Niemann-Pick disease type C. *Curr. Biol.* 11, 1283-1287.

---

## **Intravenous, non-viral gene therapy of the brain**

William Pardridge M.D. - [wpardridge@mednet.ucla.edu](mailto:wpardridge@mednet.ucla.edu)

*Professor of Medicine - UCLA-Medicine/Endocrinology, Los Angeles, California*

The genes causing inherited brain disease are mostly known, yet no brain disease has yet responded to gene therapy. The limiting problem is not gene discovery, but rather gene delivery. The direct injection of a gene into the brain delivers the gene to <1% of brain cells. The delivery of therapeutic genes to the majority of cells in the brain is possible, but must involve a trans-vascular delivery route following an intravenous injection. Virtually every neuron is perfused by its own blood vessel, but this blood vessel wall is impermeable to gene medicines, owing to the presence of the blood-brain barrier (BBB). Because the gene medicines cannot cross the BBB, there is little hope of treating global brain diseases with gene therapy unless the BBB problem is solved.

It is now possible to safely target non-viral gene medicines across the BBB following a simple intravenous administration. This is accomplished with the use of molecular Trojan horses (MTH), which cross the BBB via endogenous transport systems that are expressed within the capillary endothelium of brain that forms the BBB. By using the natural portals of entry into the brain, the MTH carries with it any attached drug or gene. Genes are ferried across the BBB by packaging the gene inside a nanocontainer, called a pegylated immunoliposome, or PIL, and decorating the surface of the PIL with the MTH. Genetically engineered MTHs suitable for use in humans are now available. The global delivery of an exogenous gene encoding for beta-galactosidase to the adult Rhesus monkey brain following an intravenous injection of a non-viral formulation of the gene has been demonstrated. Application of the PIL gene targeting technology in an experimental brain cancer model resulted in a 100% increase in survival time, and application to experimental Parkinson's disease resulted in a 100% normalization of striatal tyrosine

hydroxylase enzyme activity. The PIL gene targeting technology can be used to deliver to the human brain non-viral gene medicines for the chronic treatment of inborn errors of brain metabolism.

---

## **Substrate Reduction Therapy**

Fran Platt Ph.D. - [francis.platt@bioch.ox.ac.uk](mailto:francis.platt@bioch.ox.ac.uk)

*Lister Fellow, Oxford Glycobiology Institute, Department of Biochemistry, University of Oxford, Oxford, United Kingdom*

The glycosphingolipid lysosomal storage diseases result from defects in glycosphingolipid catabolism. They are progressive disorders, the majority of which involve storage and pathology in the CNS. A new approach to treatment is substrate reduction therapy (SRT), using small molecules to reduce the rate of glycosphingolipid biosynthesis, to offset the catabolic defect. This strategy has been evaluated in mouse models of the GM2 gangliosidosis (Tay-Sachs and Sandhoff disease), GM1 gangliosidosis and Fabry disease. The results of these studies and the comparative analysis of two different drugs for substrate reduction therapy will be presented. The implications of these findings for neuronopathic forms of Gaucher diseases will be discussed.

---

## **Role of Inflammation in the Neurodegeneration of Sandhoff disease**

Richard L. Proia Ph.D. - [rp36y@nih.gov](mailto:rp36y@nih.gov)

*Chief, Genetics of Development and Disease Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland*

Sandhoff disease is a lysosomal storage disorder caused by an inherited deficiency of b-hexosaminidase. As a consequence, glycosphingolipids —substrates for the enzyme – accumulate in cells and trigger an intense neurodegenerative course. Studies on a mouse model of Sandhoff disease and on human samples have revealed that a vigorous inflammatory response precedes the period of neurodegeneration. The inflammatory response is dominated by macrophages that

enter the central nervous system from the blood stream. We have recently obtained evidence that demonstrates that the macrophages entering the central nervous system significantly contribute to the neurodegeneration in this disorder.

---

## **The Use of Bone Marrow-Derived Cells For The Treatment of Brain Disease in a Mouse Model of Niemann-Pick Disease**

Edward Schuchman Ph.D. - [edward.schuchman@mssm.edu](mailto:edward.schuchman@mssm.edu)

*Professor and Vice Chairman for Research, Mount Sinai School of Medicine  
Department of Human Genetics, New York, NY*

A mouse model of acid sphingomyelinase (ASM)-deficient Niemann-Pick disease (i.e., Types A & B NPD) has been used to evaluate bone marrow-mediated therapies for the human disorder. Intravenous transplantation of normal bone marrow cells or bone marrow cells genetically engineered to overexpress ASM led to marked correction of visceral organ disease in the treated mice, and partial improvements in the central nervous system (CNS). Therefore, we next evaluated the ability of normal, bone marrow-derived mesenchymal stem cells (MSC) to alter the progression of CNS disease in the NPD mouse following direct, intracerebral transplantation. About half of the treated mice subjected to this procedure had improved rotarod performance compared to untreated mice, and an improved lifespan. Histological analysis of the CNS at various times post-transplant revealed the positive effects of this gene therapy procedure on lipid storage and Purkinje cell dropout. We next combined the intracerebral MSC transplant procedure with intravenous transplantation of retrovirally-transduced bone marrow cells. We found that the two procedures were synergistic and led to marked CNS improvements in the treated animals. However, beginning at about 24 weeks post-transplant, an immunologic response to the expressed human enzyme occurred, followed by a slow decline in neurologic function. We conclude that bone marrow-derived MSC may be very useful for cell-based therapies of Type A NPD and other neurodegenerative lysosomal storage disorders, particularly because this same cell type may be used to treat both visceral organ and CNS complications associated with many of these diseases.

---

## **Gene or cell based enzyme replacement therapy leads to widespread correction of storage pathology in a mouse model of Niemann Pick A disease.**

Greg Stewart Ph.D. - [greg.stewart@genzyme.com](mailto:greg.stewart@genzyme.com)

*Genzyme Corporation, Framingham, Massachusetts*

Type A Niemann-Pick disease (NPA) is a fatal, neurometabolic childhood disorder caused by a genetic deficiency of acid sphingomyelinase (ASM). The lack of functional ASM results in sphingomyelin and cholesterol accumulation within the lysosomal compartment of cells throughout the brain leading to neurodegeneration. Using an ASM knockout (ASMKO) mouse model, we are evaluating enzyme replacement therapy using in vivo (viral vector) or ex vivo (neural stem cell) gene therapy.

An adeno-associated virus (AAV) encoding human ASM (AAV-ASM) was injected into the hippocampus of adult ASMKO mice at an age when significant storage pathology was present. This resulted in human ASM mRNA and protein expression in all major cell layers of the ipsilateral hippocampus for up to 26 weeks post-injection. Transduced cells were also present in the entorhinal cortex, medial septum, and contralateral hippocampus in a pattern consistent with retrograde transport of AAV. In all brain regions expressing human ASM, there was a substantial reduction of distended lysosomal pathology and an almost complete reversal of cholesterol accumulation. Injection of AAV-ASM into other brain regions, such as the striatum and cerebellum, resulted in the same pattern of local and distant (retrograde) transduction, ASM expression, and reversal of pathology.

In parallel, we are evaluating the use of neural stem cells (NSCs) as an alternative approach for providing enzyme replacement to the ASMKO brain. NSCs derived from the adult mouse brain were genetically modified by retroviral transfection to express human ASM, and transplanted into multiple regions of the ASMKO brain (100,000 cells/site). Transplanted NSCs survived, migrated, and differentiated in the host brain in an age- and region-dependent manner. Although levels of ASM expression were quite low, there was clearance of storage pathology within the ASMKO brain that closely overlapped with the distribution of gene-modified NSCs. No correction of pathology occurred if non-transduced NSCs were implanted.

Using either gene or cell-based therapy, our data show that ASM enzyme replacement therapy leads to long-lasting, widespread reversal of lysosomal

storage pathology in an ASMKO mouse model of NPA. Collectively, these results suggest that the NPA brain will be highly responsive to enzyme replacement therapy raising the prospects for future clinical trials.

---

## **Adeno-Associated Virus Vector-Mediated Gene Transfer to the Brain of Cats with Alpha-Mannosidosis.**

Charles H. Vite, DVM, Ph.D. - [vite@vet.upenn.edu](mailto:vite@vet.upenn.edu)

*Assistant Professor, Diplomate American College of Veterinary Internal Medicine, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, Pennsylvania*

A large number of single gene disorders affect the central nervous system (CNS), many of which are caused by deficient activity of a specific enzyme in a metabolic pathway. A number of methods have been developed to transfer genes into the rodent brain, but there are little data on gene transfer to the CNS of large animals. Our aims were to evaluate the distribution and transduction patterns of adeno-associated virus (AAV) vectors in the large animal brain and to evaluate the efficacy of therapy of a naturally occurring lysosomal storage disease in the cat using neurological examination, magnetic resonance imaging (MRI), biochemistry, and histology.

First, we evaluated the ability of three AAV serotypes to transduce and express the lysosomal enzyme  $\beta$ -glucuronidase (GUSB) in the normal cat brain. The human GUSB cDNA under the control of the human GUSB promoter was packaged into AAV1, 2, and 5 serotype capsids and injected into the cerebral cortex, caudate nucleus, thalamus, corona radiata, internal capsule, and centrum semiovale of 8-week-old cats. The brains were evaluated for gene expression using in situ hybridization, enzyme histochemistry, and biochemistry 10 weeks after surgery. The AAV2 vector was capable of transducing cells in the gray matter, while the AAV1 vector resulted in greater transduction of the gray matter than AAV2 as well as transduction of the white matter. AAV5 did not result in detectable transduction in the cat brain.

Based on these studies, AAV1 vectors were developed for testing in the naturally occurring feline model of  $\alpha$ -mannosidosis. An AAV1 vector carrying the normal feline  $\alpha$ -mannosidase cDNA was injected bilaterally into the sites listed above as

well as into the cerebellum of six 8-week-old affected cats. Weekly neurological examinations were performed on three untreated, six treated, and three normal cats. Imaging analyses were performed at 16 weeks of age followed by post mortem analysis by histology, in situ hybridization, and biochemical analysis of the effects of treatment. Treated cats showed remarkable improvement in clinical neurological signs and in lesions seen by MRI in white matter areas. Although gene transfer was limited to the areas surrounding the injection tracks, storage lesions were reduced throughout the brain. Thus, global improvement in neuropathology was achieved in a large mammalian brain using a clinically feasible number of injections, and mediated clinically significant improvement in the neurological syndrome.

---

## **Secondary Glycolipid Accumulation in Lysosomal Disorders**

Steven U. Walkley, D.V.M. Ph.D. - [walkley@aecom.yu.edu](mailto:walkley@aecom.yu.edu)

*Professor of Neuroscience, Pathology and Neurology, Department of Neuroscience, Department of Pathology, Sidney Weisner Laboratory of Genetic Neurological Disease, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, New York*

Whereas primary storage of glycolipids in neurons is a well recognized phenomenon in two major groups of lysosomal disorders (GM1 and GM2 gangliosidoses), there is evidence that gangliosides (e.g., GM2 and GM3) and other glycolipids also accumulate in a wide array of other disorders of the lysosomal system, including Niemann-Pick types A (NPA) and C (NPC) diseases, most types of mucopolysaccharidosis (MPS), and even the oligosaccharide storage disease,  $\alpha$ -mannosidosis<sup>1</sup>. Importantly, the re-appearance of one of these gangliosides (GM2) in mature neurons, whether as a primary consequence of the metabolic defect (e.g., Tay-Sachs disease) or as a secondary, downstream event (e.g., the diseases listed above), has been correlated with regrowth of dendrites (“ectopic dendritogenesis”) on cortical pyramidal cells and other select neurons<sup>2</sup>. Ectopic dendrites exhibit new synapse formation and are considered deleterious to normal brain function. These findings indicate that secondary ganglioside storage is of importance in the pathogenesis of lysosomal diseases and suggest that the recycling/synthesis of glycosphingolipids (GSLs) is often perturbed as a consequence of errors in endosomal-lysosomal function. Major goals of the current studies are focused on determining why these secondary ganglioside elevations occur, how they might be linked to new dendrite growth, and whether agents designed to

control the synthesis of GSLs (substrate reduction therapy, SRT) might be useful in clinical disease management.

For the storage disease, NPC, as caused by deficiency of the NPC1 protein, we have documented intraneuronal accumulation of GM2 and GM3 gangliosides as well as free cholesterol<sup>3</sup> and recently, through the use of double genetic mutants, have also revealed that the latter storage is dependent on that of gangliosides<sup>4</sup>. The use of imino sugars for SRT has further shown that inhibiting GSL synthesis slows disease onset and progression<sup>5</sup>. Confocal microscopy studies of NPC1-deficient neurons revealed, unexpectedly, that the two major GSL compounds stored, GM2 and GM3 gangliosides, largely reside in separate populations of vesicles, suggesting possible independent mechanisms of ganglioside sequestration<sup>6</sup>. Interestingly, NPC disease due to NPC2 protein deficiency has revealed a similar GM2-GM3 separation and indeed, parallel studies in murine models of MPS disease (types I, IIIA, IIIB, and VII) showed not only that an accumulation of free cholesterol accompanied ganglioside storage but also the same minimal co-mingling of GM2 and GM3 gangliosides<sup>7</sup>. These findings suggest that common mechanisms of ganglioside trafficking, degradation and/or synthesis are evoked in many lysosomal diseases regardless of the primary defect and that such abnormalities are routinely accompanied by cholesterol trapping. Determining how defects of endosomal-lysosomal function contribute to altered ganglioside and cholesterol expression and how this altered expression in turn impacts neuron function are key elements in understanding the complex pathogenic mechanisms underlying these diseases.

---

### **Treatment approaches for the mouse models of globoid cell leukodystrophy.**

David A. Wenger Ph.D. - [david.wenger@jefferson.edu](mailto:david.wenger@jefferson.edu)

*Professor of Neurology/Biochemistry/Molecular Pharmacology, Director of Lysosomal Disease Testing Laboratory, Jefferson Medical College, Department of Neurology, Philadelphia, Pennsylvania*

Globoid cell leukodystrophy (GLD) or Krabbe disease is a neurodegenerative disorder caused by the deficiency of the lysosomal enzyme galactocerebrosidase (GALC). This leads to the accumulation of psychosine, resulting in the death of myelin-producing-cells. Twitcher (twi), the naturally occurring mouse model of this disease, and our newly generated transgenic (tg) mice with milder symptoms and a slightly longer life span provide excellent models for evaluating therapeutic

strategies. Bone marrow transplantation (BMT) of young twi mice can prolong their lives to about 100 days from 40 days in untreated mice. In an attempt to improve the outcome of BMT, we have subjected our tg mice to BMT following pretreatment of BM cells with IGF-1. We have transplanted more than 80 tg mice. Their lifespan was extended from 50 days up to one year. Although the long-living mice still died with typical neurological symptoms, their psychosine levels were 16-40 pmol/mg prot compared to ~ 200 in 50-day-old untreated mice. Pretreatment of BM cells with IGF-1 (50 ng/ml) prior to transplantation reduced the mortality from 80% to about 50% within the first 50 days post-transplantation. In addition, we have cloned mouse GALC cDNA in pZAC2.1, a type-2 adeno-associated viral vector (AAV), packaged in AAV-1, AAV-2 and AAV-5 serotypes. In vitro transduction of mouse neural cells and skin fibroblasts with these vectors showed a 5 to 6-fold increase in GALC activity. Subsequently, these viral vectors were injected intra-ventricularly or intra-parenchymaly into the brains of newborn twi mice. Frozen sections were stained with anti-GALC antibody, and transduced cells were detected by their bright perinuclear staining. Analysis of different brain regions 2 wks after injection revealed up to a 100-fold increase in GALC activity near the site of injection. More than one approach may be needed to prevent and correct the pathology seen in both the CNS and PNS in this disease.

---

## **NEUROPATHOLOGY OF GAUCHER DISEASE: CALCARINE CORTEX LAYER 4B AND HIPPOCAMPAL CA4-2 REGION PATHOLOGY WITH SYNUCLEIN POSITIVE, LEWY BODY-LIKE INCLUSIONS**

Lt. Col. Kondi Wong M.D. USAF MC - [kondi.wong@lackland.af.mil](mailto:kondi.wong@lackland.af.mil)

*Chief, Division of Neuropathology, Wilford Hall Medical Center, Lackland, AFB Texas*

We evaluated the neuropathology of 14 patients with Gaucher disease (GD) to find specific regional pathologic changes that would further elucidate pathologic mechanisms in Gaucher disease, and common mechanisms among GD phenotypes. The patients that we studied included seven that had type 1 GD, three that had type 2 GD, and four that had type 3 GD. Neuropathologic examination found that the cerebral cortical layer 3 and 5, Hippocampal (HCP) CA2-4, and calcarine cortex layer 4b regions had pathologic disease in all GD phenotypes, but differing in severity of disease. Specifically, the exact regions and lamina were involved with neurodegeneration predominating in type 2 and 3

disease, whereas type 1 disease had only astrogiosis. The patterns of disease suggested a precise targeting of discrete regions because the adjacent regions and lamina were spared of pathology, namely, HPC CA1, adjacent calcarine lamina 4a and 4c, and layer 4 of the cerebral cortex. The pathologic findings correlate with recent biochemical experimentation that shows increased neuronal glucocylceramide (GlcCer) in Gaucher disease augmenting, modulating and sensitizing the ryanodine receptor (RyaR), potentiating calcium induced calcium release (CICR) susceptible neurons in CA4-2 regions, while CA1 neuron calcium pools are insensitive to CICR and RyaR potentiation. Targeting of CA4-2 neurons is further supported by densely reactive antiglycosyl-ceramidase immunohistochemistry, presence of numerous synuclein reactive-Lewy body-like inclusions in two patients, and concomitant diffuse Parkinson's disease/Diffuse Lewy body disease (known to have CA3-2 disease) in 3 GD patients. The latter associations also argue for a common cytotoxic mechanism linkine aberrant glucocylceramidase activity, Gaucher disease cytotoxicity and cytotoxic Lewy body formation.

---

### **“Zavesca<sup>®</sup> in adult patients with type I Gaucher disease: implications for use in neuronopathic forms”**

Ari Zimran, M.D. - [gaucher@szmc.org.il](mailto:gaucher@szmc.org.il)

*Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel*

Gaucher disease is caused by an enzymatic defect with consequent accumulation of glucocerebroside. Classically, the disease is described in three forms: type I, known as the non-neuronopathic form which is common among Ashkenazi Jews although it is also panethnic, while the neuronopathic forms are rarer with limited ethnic predilection. Symptomatic patients may present with hepatosplenomegaly, anemia, thrombocytopenia, and skeletal or lung involvement, whereas types II and III exhibit neurological features. Enzyme replacement therapy ameliorates visceral disease symptoms and signs; however, it is incapable of crossing the blood-brain barrier. Substrate reduction with *N*-butyldeoxynojirimycin (Zavesca<sup>®</sup>) is the first oral iminosugar to be developed for the treatment of glycolipid storage disorders (GLSDs). Long-term data from extension of the seminal trials with Zavesca in adult patients with type I Gaucher disease demonstrates efficacy in reducing organomegaly and improving hematological parameters. The side-effects of treatment with Zavesca are initially diarrhea and weight loss in many patients, and

some with tremor. Five cases of peripheral neuropathy were also identified during the clinical studies, although in three patients there were other etiologies clearly linked to these findings. Hence, neurological assessments were performed in all patients receiving Zavesca as well as a control cohort receiving enzyme replacement therapy or no treatment at all. We present the results from these studies, and discuss their relevance to patients with neuronopathic forms of Gaucher disease. The potential for Zavesca, a small molecule capable of crossing the blood brain barrier, as a treatment for neuronopathic GLSDs will be considered.

---