

# Promising Therapies for Niemann-Pick Type C Disease

Organizers: Danilo A. Tagle and Steven U. Walkley  
Hilton Rockville Hotel and Executive Meeting Center  
Rockville, Maryland  
June 3-4, 2010

## Abstracts of Presentations

### **Session Title: Overview of existing therapies for NPC disease: strategies and challenges for discovery of new therapies**

**Daniel Ory, M.D.**

**Daniel Ory, M.D.**

Professor of Medicine, Cell Biology and Physiology  
Washington University School of Medicine  
St. Louis, MO

**Presenter: Steven U. Walkley, D.V.M., Ph.D.**

Professor of Neuroscience  
Albert Einstein College of Medicine  
Bronx, NY

Niemann-Pick type C1 (NPC1) disease is a rare, progressive, neurodegenerative disorder characterized by accumulation of cholesterol and other lipids in the viscera and central nervous system. Affected individuals typically present in early childhood with ataxia and progressive impairment of motor and intellectual function, and usually die in adolescence. There are currently no FDA-approved therapies for this disorder. Major barriers to the development of more effective treatments for NPC have been its rare disease status, which has not attracted significant investment from the pharmaceutical industry, and the lack of outcome measures to evaluate efficacy of therapy in clinical trials. Building on recent advances in our understanding of NPC1 protein biology and in the pathogenesis of NPC disease, the NPC research community is collectively engaged in discovery of small molecule agents with disease-modifying potential and evaluation of these agents in animal models of disease and in human clinical trials. In a parallel development, human samples obtained through an NIH-sponsored natural history study have enabled discovery of biomarkers, which are being validated in animal models and humans and, ultimately, will provide long sought-after biochemical metrics that can serve as surrogate endpoints for evaluation of novel therapeutics in clinical trials.

**Session Title: High-throughput screens for discovery of small molecules with NPC disease-modifying potential**

**Yiannis A. Ioannou, Ph.D., Fannie W. Chen, Chunlei Li**

**Presenter: Yiannis Ioannou, Ph.D.**

Associate Professor

Department of Genetics and Genomic Sciences

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New York, NY

In NPC disease, cells are unable to metabolize LDL-derived cholesterol due to its accumulation in the late endosomal/lysosomal (E/L) system. During our studies of this disease, we discovered that upregulation of the small GTPase Rab9 in NPC1 cells resulted in a correction of their cellular phenotype, including clearance of accumulated cholesterol from their E/L system. Specifically, the transport of cholesterol from the E/L system to the plasma membrane is restored with a concomitant increase in cholesterol esterification at the endoplasmic reticulum. Furthermore, Rab9 overexpression affects the function of the E/L system independently of the NPC1 protein, in effect “suppressing” the NPC1 defect. These results suggested the existence of alternative endogenous therapeutic targets that could be modulated to reverse the NPC1 disease phenotype. The results also identified increasing Rab9 expression as a potential treatment modality for this disorder. These observations have provided the rationale for identifying compounds that can increase the expression of endogenous Rab9 in NPC1 cells.

We developed a Rab9 promoter assay by engineering the human Rab9 promoter to drive the expression of firefly luciferase in stable cell lines. Using these cells, we screened libraries of drug-like compounds (~100,000 compounds) and identified more than 80 compounds that are able to increase the transcriptional activity of the Rab9 promoter. Of these, 25 compounds exhibit biological activity at nanomolar concentration and can significantly increase Rab9 transcriptional activity with minimal cellular toxicity. Also, these compounds are able to correct the transport block of NPC1 patient cells and significantly increase LDL-derived cholesterol esterification.

These results confirm the validity of this approach and provide the rational and preliminary data for using medicinal chemistry to modify the identified compounds to improve their activity, toxicity, and brain delivery.

**Session Title: Evaluation of candidate compounds in animal models of NPC disease**

**Steven U. Walkley, D.V.M., Ph.D.**

**Presenter: Steven U. Walkley, D.V.M., Ph.D.**

Professor of Neuroscience

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The functions of NPC1 and NPC2 proteins, as well as the details of complex disease cascades set in motion by their absence in brain and other organs, remain poorly understood. Yet translational studies directed at therapeutic intervention are critical to pursue, and the availability of well-characterized genetic models in animals provides an ideal opportunity for progress. Candidate compounds are being identified through basic

research discoveries and through high-throughput screens using established cell systems. Although the ideal therapeutic agent for NPC disease would intervene at the outset of the disease cascade (by directly replacing or rescuing NPC1 or NPC2 proteins), drugs with impact on later, downstream features of disease (intracellular storage of cholesterol or glycosphingolipids, neuroaxonal dystrophy, neurofibrillary tangle formation, inflammation, neuron death, etc.) also might provide significant benefit, particularly when administered in combination. Current studies are directed at identifying and prioritizing candidate drugs, which are then tested individually in short and/or long-term trials in *Npc1*<sup>-/-</sup> mice. The most promising compounds emerging from these trials are then further evaluated in the feline NPC model and as combinatorial therapies in the mouse model. Evaluations include behavioral, genetic, morphological, biochemical, electrophysiological, and appropriate imaging techniques, with drug administration and evaluation being tailored to the specific agents under investigation and models used. Tissues/fluids are also harvested for biomarker analysis. The goal here is to use a multidisciplinary, multi-investigator, and highly collaborative research approach to leverage every resource towards the rapid development of an efficacious therapy for NPC disease in humans.

**Session Title: Biomarker development and clinical aspects of Niemann-Pick type C disease: natural history/observational trials as a critical first step**

**Forbes D. Porter, M.D., Ph.D.**

**Presenter: Forbes D. “Denny” Porter, M.D., Ph.D.**

Senior Investigator, Acting Clinical Director

Eunice Kennedy Shriver National Institute of Child Health and Human Development

National Institutes of Health

Bethesda, MD

Niemann-Pick type C (NPC) disease is an autosomal recessive, neurodegenerative disorder due to impaired endosomal cholesterol and lipid transport. The age of onset varies, with cases manifesting from infancy to adulthood. Classically, initial neurological symptoms are observed in early-to-late childhood. Symptoms and signs of NPC include prolonged neonatal jaundice, splenomegaly, and various neurological manifestations, especially ataxia, dysmetria, dysarthria, vertical supranuclear gaze palsy, and cognitive decline. Currently, there are no approved therapies for NPC. Although a number of therapeutic interventions have been suggested based on mouse studies, the absence of a universally accepted outcome measure is an impediment to the development of therapeutic trials for NPC. The heterogeneous clinical nature and variable age of onset compound the characterization of potential biomarkers.

In an effort to identify potential clinical or biochemical outcome measures, a longitudinal observational trial was initiated at NIH in August 2006. The primary goal of this trial was to identify clinical findings or biochemical markers with the potential to be used as outcome measures in a therapeutic trial. To quantify disease progression, a clinical severity scale was developed. This scale is being used to (1) quantify disease progression in NPC, (2) correlate potential biomarkers with disease status, and (3) provide a long-term outcome measure to evaluate therapeutic efficacy. To date, we have enrolled 49 subjects ranging in age from 3 months to 54 years. In addition to clinical characterization, we are collecting serum, urine, and cerebral spinal fluid for biomarker discovery. Initial studies have demonstrated increased oxysterols and markers of oxidative stress in NPC patients. Increased serum oxysterols not only have the potential to serve as an outcome measure for a therapeutic trial but also could form the basis of a screening or diagnostic test. In comparison with control subjects, NPC patients have increased levels of calbindin D in cerebral spinal fluid.

Calbindin D is a marker of Purkinje cell damage and thus might provide a surrogate marker for cerebellar dysfunction, which is a major clinical symptom in NPC.

**Session Title: Discovery of biomarkers for NPC disease**

**Daniel Ory, M.D.**

**Daniel Ory, M.D.**

Professor of Medicine, Cell Biology and Physiology  
Washington University School of Medicine  
St. Louis, MO

**Presenter: Forbes D. “Denny” Porter, M.D., Ph.D.**

Senior Investigator, Acting Clinical Director  
Eunice Kennedy Shriver National Institute of Child Health and Human Development  
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Bethesda, MD

There is a critical need for validated biomarkers in Niemann-Pick C (NPC) disease to facilitate diagnosis, to provide metrics of disease progression, and to use as outcome measures for the development of new therapies. To address these needs, an NPC natural history protocol (PI: F. Porter) was initiated at the NIH Clinical Center with the goal of providing patient samples for biomarker discovery. Broad-based efforts involving multiple laboratories have examined blood and CSF samples for NPC-disease-specific markers using fluorescent probe-based lysosomal morphometry in circulating lymphocytes, and unbiased proteomics and lipidomics. Several candidate NPC disease biomarkers have been identified, including plasma oxidized cholesterol species. Nonenzymatically formed oxidation products, or oxysterols, were increased in the plasma of all human NPC1 subjects studied and delineated a profile specific for NPC1 disease. This oxysterol profile also correlated with age of disease onset and disease severity. These cholesterol oxidation products provide the first robust blood-based biochemical markers for NPC1 disease and may prove useful for early detection of NPC1 disease and as outcome measures for clinical trials.

**Session Title: Development and implementation of a therapeutic trial to evaluate the safety and efficacy of n-acetylcysteine**

**Forbes D. Porter, M.D., Ph.D.**

**Presenter: Forbes D. “Denny” Porter, M.D., Ph.D.**

Senior Investigator, Acting Clinical Director  
Eunice Kennedy Shriver National Institute of Child Health and Human Development  
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Bethesda, MD

Niemann-Pick type C (NPC) disease is an autosomal recessive lysosomal storage disease with progressive neurodegeneration. It is characterized by intracellular accumulation of cholesterol and glycosphingolipids. The pathophysiological processes contributing to neurodegeneration in NPC have been intensively studied in NPC mouse models. Potential pathological processes include toxic effects of cholesterol or glycosphingolipid accumulation, deficient oxysterol production, peroxisomal dysfunction, mitochondrial dysfunction, perturbed

intracellular calcium homeostasis, inflammation, induction of apoptosis, deficient neurosteroid synthesis, and increased oxidative stress. The degree to which each of these pathological processes contributes to the pathology of NPC is not known; however, the multiple processes involved suggest that combinatorial therapy addressing various aspects of this disorder will be necessary.

A major impediment to the development of clinical trials for NPC has been the prior lack of outcome measures. Identifying biomarkers was a major goal of our NPC natural history trial, and as discussed in prior talks, we have identified a set of disease-specific and nonspecific markers of increased oxidative stress in NPC subjects. We thus have initiated a randomized, placebo-controlled, double-masked, cross-over trial to evaluate the safety and efficacy of N-acetylcysteine (NAC) to decrease markers of oxidative stress. NAC is a prodrug for glutathione, and glutathione plays a critical role in the cellular response to oxidative stress. The cross-over design was chosen to try to compensate for low numbers of patients and phenotypic heterogeneity and to accommodate off-label use of miglustat. The trial was initiated in September 2009, is fully enrolled at 35 patients, and will be completed in August 2010.

### **Session Title: Clinical trial perspective**

**Petra Kaufmann, M.D.**

#### **Presenter: Petra Kaufmann, M.D.**

Director, Office of Clinical Research  
National Institute of Neurological Disorders and Stroke  
National Institutes of Health  
Bethesda, MD

The Office of Clinical Research (OCR) at the National Institute of Neurological Disorders and Stroke (NINDS) supports clinical research that increases our understanding of the cause, diagnosis, treatment, and prevention of neurological diseases and translates scientific discoveries into improved therapies for people living with neurological diseases. This work includes initiatives that set the stage for successful trials such as natural history studies or the development of biomarkers and clinical outcome measures to assess disease progression. OCR has funding mechanisms for early-phase clinical trials, including phase I and phase II clinical proof-of-concept trials, as well as for phase III efficacy trials. Recognizing the special challenges in conducting clinical trials in rare diseases, OCR supports the development of innovative trial methodologies that may include adaptive designs or designs for small populations.

NINDS is planning a new initiative in the form of a clinical research network that will support clinical research staff to conduct NINDS-funded clinical research emphasizing phase II trials of novel interventions. The goal is to have a flexible network that can respond to translational opportunities as they arise in different diseases.

Once a clinical research study or trial has started, the timely recruitment of trial participant is often challenging. NINDS therefore encourages patient groups to partner with investigators in the planning and implementation of clinical trials so that the evaluation of new interventions can be accelerated. Clinical trials cannot be completed without patient participation. Novel treatments cannot be evaluated without clinical trials.

**Session Title: Drug repurposing for NPC through screening of the NCGC Pharmaceutical Collection small molecule library**

**Christopher Austin, M.D.**

**Christopher Austin, M.D.**

Director, Chemical Genomics Center  
National Human Genome Research Institute  
National Institutes of Health  
Bethesda, MD

**Presenter: Dr. Wei Zheng**

Drug repurposing – the use of a currently approved drug for a new indication – offers one of the few routes to a rapid therapeutic advance for NPC. In order to identify drugs that might ameliorate the cholesterol storage defect in NPC, we have screened the NIH Chemical Genomics Center (NCGC) Pharmaceutical Collection of >3000 small molecule drugs for activity in patient-derived fibroblast assays for lysosomal cholesterol storage. Readouts included cholesterol oxidase, filipin, lysosome size, and cell viability. Several drugs were identified that decreased multiple measures of lysosomal cholesterol storage but did not affect cellular viability. One series of compounds showed particularly significant activity in cells from NPC patients and other lysosomal storage disorders, and these compounds are now being studied in other biochemical, cellular, and mouse models of NPC, in a research consortium coordinated by SOAR and NIH.

**Session Title: *In vitro* testing of sphingomyelinase as a treatment for NPC disease**

**Frederick R. Maxfield, Ph.D., Nina Pipalia, Ira Tabas, Cecilia Devlin, Xianghai Liao, Edward Schuchman**

**Presenter: Frederick R. Maxfield, Ph.D.**

Professor  
Department of Biochemistry  
Weill Cornell Medical College  
New York, NY

Different primary lysosomal trafficking defects lead to common alterations in lipid trafficking, suggesting cooperative interactions among lysosomal lipids. However, cellular analysis of the *functional* consequences of this phenomenon is lacking. As a test case, we studied cells with defective Niemann-Pick C1 (NPC1) protein, a cholesterol trafficking protein whose defect gives rise to lysosomal accumulation of cholesterol and other lipids leading to NPC disease. NPC1 cells also develop a secondary defect in acid sphingomyelinase (SMase) activity despite a normal acid SMase gene (*SMPD1*). When acid SMase activity was restored to normal levels in NPC1-deficient CHO cells through *SMPD1* transfection, there was a dramatic reduction in lysosomal cholesterol. Two other defects, excess lysosomal bis-(monoacylglycerol) phosphate (BMP) and defective transferrin receptor (TfR) recycling, were also markedly improved. To test relevance in human cells, the acid SMase activity defect in fibroblasts from NPC1 patients was corrected by *SMPD1* transfection or acid SMase enzyme replacement. Both treatments resulted in a dramatic reduction in lysosomal cholesterol. These data show that correcting one aspect of a complex lysosomal lipid storage disease can reduce the cellular consequences even if the primary genetic defect is not corrected. Supported by the NIH.

## **Session Title: Calcium modulators and anti-inflammatory therapies**

**Emyr Lloyd-Evans, Ph.D.**

**Presenter: Emyr Lloyd-Evans, Ph.D.**

Department of Pharmacology  
University of Oxford  
Oxford, England

Niemann-Pick type C (NPC) disease is a complex neurodegenerative lysosomal lipid storage disorder that occurs at an estimated frequency of 1:150,000 live births. The biochemical and cellular features of NPC disease include the accumulation of several classes of lipids (cholesterol, sphingomyelin, sphingosine, and glycosphingolipids) and a block in late endosome-lysosome fusion resulting from a unique acidic store calcium defect that we recently identified (1).

Each step in the pathogenic cascade represents a potential clinical intervention point. For example, we have found that compensating for lack of calcium release from the acidic store using the weak SERCA antagonist curcumin corrects the cellular defects found in NPC disease cells and improves function and life span in the NPC1 mouse. Added benefit can be achieved if curcumin is combined with miglustat.

In common with other neurodegenerative diseases, activation of the innate immune system occurs in NPC and contributes to pathogenesis. Targeting inflammation using nonsteroidal anti-inflammatory drugs was found to be of benefit in the NPC1 mouse (2).

These studies suggest that small molecule therapeutics targeting the calcium defect and inflammation merit evaluation as potential disease modifiers/adjunctive therapies in patients with NPC disease.

(1) Lloyd-Evans et al, 2008, Nat Med, 14, 1247–1255.

(2) Smith et al, 2009, Neurobiol Disease, 36, 242–251.

## **Session Title: Imatinib (Gleevec) and other c-Abl inhibitors for treatment of Niemann-Pick type C disease**

**Silvana Zanlungo, Ph.D.**

**Presenter: Silvana Zanlungo, Ph.D.**

Departamento de Gastroenterology  
Pontificia Universidad Catolica de Chile  
Santiago, Chile

We have previously reported that the c-Abl/p73 proapoptotic pathway is activated in brains of NPC mice and that its inhibition by imatinib mesylate (Gleevec) reduces neuronal apoptosis and increases the survival of NPC mice. This neuroprotection induced by imatinib also was found in *in vitro* NPC neuron models. We also have analyzed the *in vivo* effect of more potent c-Abl inhibitors available in the market, such as dasatinib and nilotinib, in NPC mice. We found that tenfold less dasatinib protects against neurodegeneration in cultured NPC neurons, but it shows only a moderate neuroprotective effect in NPC mice. In contrast, a 100-fold lower dose of nilotinib shows body weight and locomotor function improvement similar to imatinib in NPC mice.

When exploring the upstream inductors of c-Abl/p73 in NPC disease, oxidative stress appears to be a key triggering stimulus connecting cholesterol accumulation with activation of this signaling pathway in NPC neurons. Consistent with this finding, antioxidants such as NAC decreased c-Abl/p73 induction and improved cell survival in cultured NPC neurons.

In this session, we will discuss the pros and cons of c-Abl as a therapeutic target in NPC disease, the relative usefulness of the currently available c-Abl inhibitors, and the potential use of antioxidants in combination with c-Abl inhibitors for treatment of NPC patients.

Supported by grants from the Ara Parseghian Medical Research Foundation, Fondo Nacional de Desarrollo Científico y Tecnológico (FONDECYT) (grant #1080221 to A.R.A. and #1070622 to S.Z.), and Hadley Hope Fund.

## **Session Title: Imino sugar-based inhibition of glucosylceramide synthase for treating Niemann-Pick type C disease**

**John Marshall**

**Presenter: John Marshall**

Principal Scientist  
Genzyme Corporation  
Framingham, MA

Neuropathic glycosphingolipid storage disorders may benefit from substrate reduction therapy (SRT) by using an inhibitor of glucosylceramide synthase (GCS) to decrease the synthesis of glucosylceramide and related glycosphingolipids (GSLs). NB-DNJ (miglustat) is an imino sugar-based inhibitor of GCS already approved for treatment of Niemann-Pick type C disease. Genz-529468 (MZ-21) is a more potent imino sugar-based inhibitor of GCS that is also a blood-brain barrier permeant and was used to evaluate this therapeutic concept in mouse models of Niemann Pick type C and Sandhoff disease. Treatment resulted in a delay in the loss of motor function and coordination and an extension in longevity (more than 25% greater than untreated mice). Analysis of glycosphingolipids demonstrated, as expected, inhibition of GSL accumulation in the liver. However, analysis of brain revealed a slight increase in gangliosides over the untreated controls and, more surprisingly, a tenfold to 20-fold increase in the level of glucosylceramide. We suspect that the elevated glucosylceramide level in the brain was due to secondary inhibition of the nonlysosomal glucosylceramidase, gba2. This observation suggests that the survival benefit may not necessarily be due to substrate reduction. Currently, our efforts are directed at understanding the basis for the improved function in Genz-529468-treated NPC and Sandhoff mice.

## **Session Title: Clinical studies of miglustat in Niemann-Pick type C disease**

**Marc C. Patterson M.D.**

**Marc C. Patterson, M.D.**

Chair, Division of Child and Adolescent Neurology  
Mayo Clinic  
Rochester, MN



**Presenter: Dr. Olivier Morand**

Actelion Pharmaceuticals

In the 1990s, it was shown that the administration of miglustat to mice with NPC delayed the onset of symptoms and prolonged survival. This was the first evidence in an animal model of NPC that any therapy could modify the course of the illness. A prospective controlled clinical trial was planned based on these findings. At the time, no published natural history data were available and there were no validated biomarkers for this disease. The initial trial recruited 30 patients older than 12 years with biochemically proven NPC. Patients were randomized 2:1 to receive miglustat versus standard therapy. No placebo was used, as it was expected that miglustat would produce gastrointestinal symptoms in the majority of patients, and an active placebo would have been unethical.

Subsequently a pediatric substudy involving 12 children between 4 and 12 years was added to the main study; this substudy had no direct control group. Based on limited published data and experience at the National Eye Institute, horizontal saccadic eye movement velocity alpha was selected as the primary outcome measure, with a number of secondary outcome measures.

The first 12 months of the study found evidence suggesting a beneficial effect of miglustat in the treatment group, although the study was underpowered and did not achieve statistical significance in the primary analysis. Subsequent followup studies suggested that the agent is safe when used in the long term. A retrospective study of patients receiving miglustat outside the clinical trials also provided data suggesting evidence of benefit, although this study has been criticized for methodological flaws.

**Session Title: Treatment of NPC mice with cyclodextrin: a closer look at effects on the CNS**

**Cristin Davidson, Nafeeza Ali, Steven U. Walkley, D.V.M., Ph.D.**

**Presenter: Cristin Davidson**

Graduate Student

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Bronx, NY

We and others recently discovered the ameliorating effects of 2-hydroxypropyl-beta-cyclodextrin (HPBCD) on Niemann-Pick type C (NPC) disease. Our current studies aim to elucidate the mechanism of action of HPBCD and understand the effects of dose and route of administration. To determine whether HPBCD can prevent *and* reverse storage in NPC disease, we administered the compound for 3 weeks to NPC mice starting at postnatal day 28. We found that HPBCD prevented additional accumulation and partially reversed existing cholesterol and ganglioside storage. We also investigated different routes of administration and doses, including subcutaneous bolus injections and slow, chronic infusion into the intracerebral ventricular (ICV) system of NPC mice at 4,000 or 8,000 mg cyclodextrin/kg. These short-term studies did not suggest greater reductions in cholesterol or ganglioside accumulation with ICV administration. In addition, we treated mice lacking both NPC1 and NPC2 proteins with HPBCD and found benefit equivalent to that of either treated single mutant. This finding suggests that HPBCD may either replace the normal transport mechanism carried out by the NPC proteins or work through a more generic mechanism. However, administration of HPBCD to other

lysosomal disease mouse models exhibiting cholesterol and ganglioside accumulation did not appear to affect storage, arguing that the mechanism of HPBCD may be specific to NPC disease. Finally, we have tested several different forms of cyclodextrin in NPC1 mice and discovered differences in effectiveness of preventing/reducing storage, with the order of most to least effective being HPBCD, methyl-beta-CD > sulfobutylether-gamma-CD > sulfobutylether-beta-CD > 2-hydroxypropyl-alpha-CD, sulfobutylether-alpha-CD.

**Session Title: Mechanism of action of cyclodextrins in reversing cholesterol transport defects in Niemann-Pick type C disease**

**John M. Dietschy, M.D.**

**Presenter: John M. Dietschy, M.D.**

Professor

Department of Internal Medicine

University of Texas Southwestern Medical Center

Dallas, TX

Niemann-Pick type C (NPC) disease is one of a number of disorders in which the underlying metabolic defect is abnormal accumulation of either cholesterol (C) or cholesteryl esters (CEs). The severity of the disease in organs like liver, lung, and CNS is proportional to the amount of sterol that accumulates in that particular tissue, and interventions that prevent this accumulation prevent the disease. Administration of cyclodextrin (CYCLO) rapidly overcomes the C transport defect seen in NPC1 and NPC2 disease and allows the sterol to move to the cytosolic compartment of cells, to be transported to the liver, and ultimately to be excreted from the body as bile acid. The ED<sub>50</sub> for this effect equals ~300 mg/kg in most organs. However, the value in kidney, which is only ~30 mg/kg, is much higher in the CNS. The ED<sub>50</sub> value for the lung is infinitely high. This ED<sub>50</sub> value for the CNS is much lower when the CYCLO is administered directly into the brain. The acute or continuous administration of cyclodextrin into the CNS normalizes cholesterol metabolism and prevents neurodegeneration. To bring about these changes, the particular CYCLO must interact with C, but it need not bring about solubilization into the bulk-water phase. After administration of appropriate doses at appropriate intervals, the pools of C in nearly every organ are maintained at normal levels and disease is prevented. Only in lung is the abnormal C metabolism resistant to CYCLO therapy. Consequently, whereas liver and CNS disease can be prevented, pulmonary disease progresses.

**Session Title: Intrathecal and subcutaneous cyclodextrin therapy of feline Niemann-Pick type C disease**

**Charles Vite, D.V.M., Ph.D.**

**Presenter: Charles Vite, D.V.M., Ph.D.**

Assistant Professor of Veterinary Neurology

School of Veterinary Medicine

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Philadelphia, PA

The feline NPC model has a spontaneously occurring missense mutation in NPC1 (2864G-C) and has clinical, neuropathological, and biochemical abnormalities similar to those present in juvenile-onset patients, making this model homologous to the most common disease form seen in human patients. We completed a miglustat

therapy trial and, by fall, will complete a hydroxypropyl $\beta$ -cyclodextrin dose-response therapy trial (HP $\beta$ CD; subcutaneous administration of 1,000, 4,000, or 8,000 mg/kg; intrathecal administration of 120 mg). We identified that intrathecal administration of HP $\beta$ CD ameliorated all clinical aspects of neurological disease at least up to 24 weeks of age (an age when untreated cats die) but had no effect on hepatic disease. We identified that while subcutaneous therapy with HP $\beta$ CD at all doses ameliorated liver disease, only 8,000 mg/kg substantially affected neurological disease but also resulted in early death due to pulmonary toxicity. Finally, we identified a dose-related toxic effect of HP $\beta$ CD on hearing function that had not been described in any other species.

### **Session Title: Cyclodextrin and the blood-brain barrier**

**David J. Begley, Ph.D.**

**Presenter: David J. Begley, Ph.D.**

Blood-Brain Barrier Group  
Kings College London  
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The CNS penetration of cyclodextrin was investigated using standard methods. Transcardiac brain perfusion for 2 minutes with tracer 2-hydroxy- $^{14}$ C-propyl- $\beta$ -cyclodextrin (CD) gave a volume of distribution ( $V_d$ ) for the compound of  $0.113 \pm 0.010$  ml/g of brain in npc1+/+ mice and  $0.071 \pm 0.002$  ml/g of brain in npc1-/- mice. This compares with brain volumes of distribution for  $^{14}$ C-sucrose of  $0.039 \pm 0.006$  ml/g in npc1+/+ mice and  $0.047 \pm 0.005$  ml/g in npc1-/- mice for the same time period. Sucrose is a marker of brain vascular space, and it would appear that the  $V_d$  of CD is larger than the expected vascular space. However, when the experiments are extended to 4 minutes, no further increase in the  $V_d$  for CD was observed as would be anticipated if CD was crossing the blood-brain barrier (BBB) and entering brain. The  $V_d$  for cyclodextrin in the npc1+/+ mice could be reduced by ~25% by the addition of 2mM CD to the perfusate, suggesting that  $^{14}$ C-CD is probably bound nonspecifically to the glycocalyx of the cerebral endothelial cells and can be displaced by an excess of nonradiolabelled CD.

A multitime-point regression analysis of BBB penetration in npc1+/+ and npc1-/- mice over 1 hour also was conducted following intraperitoneal administration of labelled compound in tracer quantities or combined with unlabelled carrier cyclodextrin at doses of 4,000 mg/kg, producing effective plasma levels of CD of ~1mM. These studies also revealed no increase in brain  $V_d$  over a 1-hour exposure, again suggesting a possible surface binding with no translocation of CD across the BBB. There were no discernable differences in CD penetration of the BBB in 6- to 8-week-old mice and 7-day-old mice.

This work was supported by a research grant from the Hadley Hope Fund and the Addi and Cassi Fund.

### **Session Title: Cyclodextrin overcomes deficient lysosome-to-endoplasmic reticulum transport of cholesterol in Niemann-Pick type C cells**

**Lina Abi-Mosleh, Ph.D., Rodney E. Infante, Arun Radhakrishnan, Joseph L. Goldstein, Michael S. Brown**

**Presenter: Lina Abi-Mosleh, Ph.D.**

Department of Molecular Genetics  
University of Texas Southwestern Medical Center

A handoff model has been proposed to explain the egress from lysosomes of cholesterol derived from receptor-mediated endocytosis of LDL. Cholesterol is first bound by soluble Niemann-Pick C2 (NPC2) protein, which hands off the cholesterol to the N-terminal domain of membrane-bound NPC1. Cells lacking NPC1 or NPC2 accumulate LDL-derived cholesterol in lysosomes and fail to deliver LDL cholesterol to the endoplasmic reticulum (ER) for esterification by acyl-CoA acyltransferase (ACAT) and for inhibition of sterol regulatory element-binding protein cleavage. Here, we support this model by showing that the cholesterol transport defect in NPC1 mutant cells is restricted to lysosomal export. Other cholesterol transport pathways appear normal, including the movement of cholesterol from the plasma membrane to the ER after treatment of cells with 25-hydroxycholesterol or sphingomyelinase. The NPC1 or NPC2 block in cholesterol delivery to the ER can be overcome by 2-hydroxypropyl-beta-cyclodextrin, which leads to a marked increase in ACAT-mediated cholesterol esterification. The buildup of cholesteryl esters in the cytosol is expected to be much less toxic than the buildup of free cholesterol in the lysosomes of patients with mutations in NPC1 or NPC2.

**Session Title: Modulation of cholesterol trafficking by cyclodextrin in NPC1-deficient mouse neurons**

**Jean E. Vance, Ph.D., and Kyle B. Peake**

**Presenter: Jean E. Vance, Ph.D.**

Professor, Department of Medicine  
University of Alberta  
Edmonton, Alberta, Canada

In the neurodegenerative disorder Niemann-Pick type C (NPC) disease, the intracellular trafficking of cholesterol in all cells, including cells of the CNS, is impaired, resulting in the sequestration of unesterified cholesterol and other lipids in late endosomes/lysosomes (LE/L). Cholesterol is synthesized in the CNS by neurons and glial cells, primarily astrocytes. We have investigated defects in cholesterol trafficking/homeostasis in neurons and glial cells isolated from NPC1-deficient mice. Astrocytes generate and secrete lipoproteins that contain cholesterol and apo E, a ligand for receptors of the low-density lipoprotein receptor family. Thus, cholesterol can be delivered to neurons from astrocytes via endocytosis of apo E-containing lipoproteins. We have shown that, in neurons, the cholesterol content of cell bodies is increased by NPC1 deficiency but is decreased in distal axons. Our results are consistent with the idea that a deficiency of cholesterol in distal axons alters synaptic vesicle recycling, thereby impairing synaptic transmission. Recent studies by Dietschy and coworkers in NPC1-deficient mice have demonstrated that cyclodextrin, a cholesterol sequestering agent, increases the survival and improves neurological outcomes of these mice. We have used primary cultures of neurons and glial cells from NPC1-deficient mice to investigate the mechanism(s) underlying the beneficial effects of cyclodextrin. Our studies indicate that a low dose of cyclodextrin releases the sequestered cholesterol from the LE/L of NPC1-deficient neurons and glia, reduces the rate of cholesterol synthesis, and partially restores normal cholesterol homeostatic mechanisms.

**Session Title: Cellular mechanism of action of cyclodextrins in NPC cells**

**Frederick R. Maxfield, Ph.D., Anton I. Rosenbaum, J. David Warren**

**Presenter: Frederick R. Maxfield, Ph.D.**

Professor

Department of Biochemistry

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New York, NY

We examined the mechanism by which treatment of cells with cholesterol-chelating cyclodextrins can correct the NPC defect. We show that decreased levels of cholesterol accumulation are maintained for several days after removal of cyclodextrin from the culture medium. This finding suggests that endocytosed cyclodextrin can reduce the cholesterol storage by acting from inside endocytic organelles rather than by removing cholesterol from the plasma membrane. To test this further, we incubated both NPC1 and NPC2 mutant cells with cholesterol-loaded cyclodextrin for 1 hour followed by chase in serum-containing medium. Although the cholesterol content of the treated cells increased after the 1-hour incubation, the cholesterol levels in the storage organelles were later reduced significantly. We covalently coupled cyclodextrin to fluorescent dextran polymers. These cyclodextrin-dextran conjugates were delivered to cholesterol-enriched lysosomal storage organelles and were effective at reducing the cholesterol accumulation. These findings suggest that cyclodextrin-mediated enhanced cholesterol transport from the endocytic system can reduce cholesterol accumulation in cells with defects in either NPC1 or NPC2.

Supported by NIH and the Ara Parseghian Medical Research Foundation.

**Session Title: Mechanism of sterol transport by cyclodextrin**

**Leslie McCauliff and Judy Storch, Ph.D.**

**Presenter: Leslie McCauliff**

Graduate Student

Department of Nutritional Sciences

Rutgers University

New Brunswick, NJ

We examined the regulation and mechanism of sterol transport by cyclodextrin (CD) using *in vitro* model systems and fluorescence spectroscopy and NPC2-deficient fibroblasts. We demonstrate that cholesterol transport from the lysosomal cholesterol-binding protein NPC2 to three different species of CD occurs via aqueous diffusional transfer, which is very slow; the rate limiting step appears to be dissociation of cholesterol from NPC2, suggesting that specific interactions between NPC2 and CD do not occur. In contrast, the transfer rate of the fluorescent cholesterol analogue dehydroergosterol (DHE) from CD to phospholipid membranes is very rapid and is directly proportional to the acceptor membrane concentration. In addition, CD dramatically increases the rate of sterol transfer between membranes, with rates that approach those mediated by NPC2. These results suggest that sterol transfer from CD to membranes occurs by a collisional transfer mechanism, involving direct interaction of CD with membranes.

Marked enhancement of sterol transport by CD also was found in cultured cells, where addition of CD rapidly rescued the cholesterol accumulation phenotype of NPC2-deficient fibroblasts, as assessed by filipin staining.

Thus, the recent observations of CD efficacy in mouse models of NPC disease are likely the result of CD enhancement of cholesterol transport between membranes, with rapid sterol transfer occurring during CD-membrane interactions.

**Session Title: Are all commercial hydroxypropyl-beta-cyclodextrins the same?**

**Lajos Szente, Ph.D., D.Sc.**

**Presenter: Lajos Szente, Ph.D., D.Sc.**

Chief Executive Officer

CycloLab, Ltd.

Budapest, Hungary

2-hydroxypropyl-beta-cyclodextrin (HPBCD) has long been used as a potent solubilizer for water-insoluble drugs. The well-tolerated solubilizer excipient is approved in a number of parenteral pharmaceutical dosage forms (e.g., Sporanox<sup>®</sup>, Dyloject<sup>®</sup>). This cyclodextrin derivative is a composite, isomeric mixture material, X-ray amorphous, highly soluble, and noncrystallizable in water. The solubilization power is based on its inclusion complex forming potency, a noncovalent interaction between drug and subnanometer-sized HPBCD cavity. HPBCD “enjoys” broad acceptance criteria in the U.S. Pharmacopeia and European Pharmacopoeia in terms of degree of substitution (DS). The acceptance range is between DS = 2.8 and DS = 10.5. Both the degree and the pattern of substitution affect the physicochemical properties, solubilizing potency, aggregation, and other functional properties of the HPBCD products.

The presentation attempts to evaluate in detail the role of DS and substitution pattern on the functional properties (solubilization effect, solution clarity/precipitation, cholesterol mobilization, aggregation tendency, etc.) of HPBCDs of different origin with low, medium, and high DS, all satisfying pharmacopoeia requirements. Tailored analytical methods (HPLC/MS/MS, MALDI, <sup>13</sup>C <sup>1</sup>H NMR) will illustrate, together with cholesterol solubilization results, the remarkable differences between commercial HPBCD products. Strengths and weaknesses of the applied pharmacopoeia and proposed analytical methods will be discussed in light of DS determination errors that often occur. CycloLab’s recommended analysis methods for HPBCD qualifications are now being considered by the European Medicines Agency and will soon be official. Based on the presented analytical and complexation/solubilization results, the most suitable types of HPBCDs for *in vivo* cholesterol mobilization and for therapeutic uses will be proposed.

**Session Title: Captisol (SBE7-β-CD) and other sulfoalkylether-modified cyclodextrins**

**James D. Pipkin, Ph.D.**

**Presenter: James D. Pipkin, Ph.D.**

Senior Director, New Product Development

CyDex Pharmaceuticals, Inc.

Lenexa, KS

CyDex Pharmaceuticals, Inc., is a specialty pharmaceutical company that develops, manufactures, and licenses modified cyclodextrins based on sulfoalkylether chemistries (SAECD). CyDex also develops its own Captisol-enabled<sup>®</sup> (SBE7-β-CD) therapeutics. The parenterally safe Captisol is currently incorporated in five FDA-approved injectable medications (Vfend, Geodon, Cerenia, Abilify, and Nexterone) and is marketed by three of

the company's licensees: Pfizer, Bristol-Myers Squibb, and Prism Pharmaceuticals. In addition, CyDex, via its technology, is supporting drug development efforts with more than 40 other companies worldwide and engages in collaborations with various institutions internationally to further understand the application of its technology. The company continues to develop and maintain patents in the United States and worldwide for its Captisol technology and Captisol-enabled products, and it maintains comprehensive FDA manufacturing and safety Drug Master Files enabling regulatory access to readily gain entry to the clinic. This brief presentation will describe the design of Captisol, its safety and clinical use, and other available derivatives.

**Session Title: Pharmaceutical application of hydroxypropyl- $\beta$ -cyclodextrin (HP $\beta$ CD)**

**Marcus E. Brewster, Ph.D.**

**Presenter: Marcus E. Brewster, Ph.D.**

Distinguished Research Fellow  
Pharmaceutical Sciences  
Johnson & Johnson  
Beerse, Belgium

HP $\beta$ CD is a useful functional excipient that has enjoyed widespread attention and use. This monomolecular complexing agent increases solubility and oral bioavailability for a number of pharmaceutically active agents through various mechanisms, including inclusion and noninclusion complex formation and supporting supersaturation. In addition, the unique features of this modified starch enable it, in and of itself, to modify lipid distribution, thereby being of potential value in a number of human maladies such as liposomal storage diseases, HIV infectivity, and hypervitaminosis A. The appropriate exploitation of HP $\beta$ CD in these disease states requires a complete and satisfactory safety, pharmacokinetic, and clinical data package together with adequate controls on the quality of the material.

This presentation will briefly present available data on the characterization and regulatory controls of the HP $\beta$ CD drug substance as overviewed in the European Pharmacopoeia monograph as well as Johnson & Johnson's collected experience with toxicity as a function of administration routes, animal species, and dose. Many of these data are collected in our Type IV DMF for the compound. Furthermore, the clinical experience with HP $\beta$ CD as an excipient in various drug products (including Sporanox<sup>®</sup>), as well as when administered alone, will be discussed. This includes studies in which IV doses as high as 16 g/d and oral doses as high as 24 g/day are evaluated. The collected data suggest that HP $\beta$ CD can be safely administered in humans over a broad range of doses supporting its investigational use in the diseases noted above.

**Session Title: Clinical experience with intravenous infusions of hydroxy-propyl-beta-cyclodextrin in identical twin patients with Niemann-Pick type C disease under individual investigational new drug applications**

**Caroline Hastings, M.D.**

**Presenter: Caroline Hastings, M.D.**

Pediatric Hematologist/Oncologist  
Children's Hospital and Research Center  
Oakland, CA

Based on animal studies of Niemann-Pick type C (NPC) disease demonstrating significant prolongation of life expectancy following the administration of hydroxyl-propyl-beta-cyclodextrin (HPBCD), parents began pursuing treatment options with cyclodextrin for their children afflicted with NPC. In November 2008, individual investigational new drug (IND) applications were filed with FDA to provide intravenous infusions of HPBCD in these children. Following negotiations with FDA on the treatment protocol, and after obtaining institutional review board approval, the twins were surgically implanted with MediPort catheters. In April 2009, the twins began receiving intravenous (IV) infusions of HPBCD. Initially, the patients received 4 days of continuous IV infusions at 80 mg/kg/day. Subsequently, doses were titrated to a level of 2,500 mg/kg/day administered twice per week in 8-hour infusions. All infusions were performed in a pediatric subspecialty clinic at Renown Regional Medical Center in Reno, NV. Frequent safety monitoring, clinical labs, and biomarker samples were obtained throughout the more than 1 year of treatment.

Overall, the IV infusions of HPBCD were well tolerated. No infusion or delayed toxicities were observed. In fact, no significant changes were seen in vital signs or clinical labs. Of interest, the 2x elevations in baseline AST were not changed following HPBCD infusions. By contrast, oxysterol biomarkers appear to have been reduced during the course of IV infusions. Pulmonary and auditory testing failed to show any deficits following nearly 1 year of HPBCD at the dose levels administered.

Although the parents believe the children are obtaining some therapeutic benefit from the IV infusions of HPBCD, the children continue to decline as evidenced by PET imaging and neurological assessment. Because recent findings suggest limited or no blood-brain barrier penetration of HPBCD following systemic administration, and direct central injection appears promising in animal studies, we intend to pursue an intrathecal route of administration under individual INDs.

## **Session Title: Resources for therapeutic development at the National Institute of Neurological Disorders and Stroke**

**William D. Matthew, Ph.D.**

**Presenter: William D. Matthew, Ph.D.**

Director, Office of Translational Research  
National Institute of Neurological Disorders and Stroke  
National Institutes of Health  
Bethesda, MD

The translation of basic science into patient therapies is a critical mission of the National Institutes of Health. Within NINDS, the Office of Translational Research (OTR) manages multiple initiatives in drug discovery and preclinical development of neurological therapeutics. These initiatives include Exploratory Projects in Translational Research, Cooperative Agreement Program in Translational Research, Anti-Convulsant Screening Program, Small Business Program, Spinal Muscular Atrophy Project, Blueprint Neurotherapeutics, CounterACT, and certain programs within the NIH Roadmap and RAID (Rapid Access to Interventional Development). An overview of OTR activities in drug discovery will be presented, and the funding mechanisms available to these programs will be discussed.



## **Session Title: FDA orphan drug development**

**Francesca Joseph, M.D.**

**Francesca Joseph, M.D.**

Medical Officer

Office of Orphan Products Development

U.S. Food and Drug Administration

Silver Spring, MD

**Presenter: Linda Ulrich**

The Food and Drug Administration has charged the Office of Orphan Products Development (OOPD) with promoting the development of products that demonstrate promise for the diagnosis and/or treatment of rare diseases or conditions. In fulfilling this task, OOPD interacts with the medical and research communities, professional organizations, academia, governmental agencies, and the pharmaceutical industry, as well as rare disease groups. OOPD administers the major provisions of the Orphan Drug Act (ODA), which provides incentives for sponsors to develop products for rare diseases. Evidence of its success lies in the more than 200 drugs and biological products for rare diseases that have been brought to market since 1983, versus fewer than 10 such products before 1983. In addition, OOPD administers the Orphan Products Grants Program, which provides funding for clinical research in rare diseases. The session will provide an overview of OOPD, orphan drug designation, and orphan drug development.

## **Session Title: Simulate 2 Design, Model 4 Approval: a mantra for enhancing the success rate of developing drugs against rare diseases**

**Jogarao Gobburu, Ph.D.**

**Presenter: Jogarao Gobburu, Ph.D.**

Director, Division of Pharmacometrics

OCP/Office of Translational Sciences

Center for Drug Evaluation and Research

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Silver Spring, MD

Developing drugs against rare diseases faces several challenges including drug screening, dose selection, patient recruitment, endpoint selection, powering a study, and potential lack of incentives. Unlike other indications, for rare diseases, industry (or other organizations) might have limited resources for clinical testing. Nevertheless, an urgent need exists to develop safe and effective treatments for this set of diseases. The presentation will demonstrate through case studies how pharmacometric modeling and simulation can improve the chances of trial success. The importance of prudent planning (front-loaded drug development) in conjunction with innovative design and analysis of clinical trials will be emphasized.

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### **Session Title: Review and regulation of small clinical trials**

**Anne Pariser, M.D.**

**Presenter: Anne Pariser, M.D.**

Associate Director for Rare Diseases  
Office of New Drugs  
Center for Drug Evaluation and Research  
U.S. Food and Drug Administration  
Silver Spring, MD

Niemann-Pick type C (NPC) disease and other inborn-errors-of-metabolism disorders with central nervous system manifestations are among the rarest of orphan disorders, with few patients available for study. Substantial challenges exist for the clinical development of new treatments for NPC. Some of those challenges include NPC's tendency to have wide phenotypic variability, its incompletely understood natural history, and a lack of available endpoints, outcome measures, tools, and instruments to assess potential therapeutic interventions. These and other factors contribute to the special challenges for the regulation and review of potential treatments for NPC.

This presentation will include an overview of requirements for entering and performing clinical trials for rare diseases, and FDA's approach to the review and regulation of small trials. Directions for the future study of potential NPC treatments that address some of the challenges presented by this disease include (1) the use of biomarkers for the diagnosis, monitoring, and assessment of disease; (2) additional disease characterization through natural history studies; (3) exploration of new outcome measures in clinical trials, such as imaging modalities, surrogates, and standardized clinical instruments; and (4) improved compartment/tissue/cellular targeting of therapies.

**Respectfully prepared by:**

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