Niemann Pick Disease Type C (NP-C Disease) is a fatal, neurodegenerative, lysosomal storage disorder. It is a rare disease with broad clinical spectrum whose pathophysiology is poorly understood. We combine whole-genome expression and cellular analyses as well as functional microbial assays of the spleen, liver, brain and blood from Balb/c NPC1<sup>-/-</sup> mice relative to those of NPC1<sup>+/+</sup> at different ages, to show significant increase in innate immune mechanisms linked to systemic elevation of neutrophils in NPC1<sup>-/-</sup> mice. Age-dependent increase of lysozyme detected in NPC1<sup>-/-</sup> mouse plasma, suggest progressive neutrophil degranulation that yields both targets and biomarkers for disease. We combine this work with a quantitative, rapid, murine neurocognitive and muscular scale to assess NP-C disease in (i) Balb/c NPC1<sup>nih</sup> mice which manifest a gene truncation and thus accelerated severe disease and (ii) Balb/c NPC1<sup>nmf164</sup> mice with a milder disease progression due to a single point mutation (D1005G) in the large cysteine-rich luminal loop of the NPC1 protein, where ~1/3<sup>rd</sup> of the identified human mutations have been found. Multiple, new therapeutic strategies are being undertaken in these models, and the outcomes on disease, plasma and tissue correlates will be presented.
Efficacy of Different Cyclodextrins in the Treatment of Niemann-Pick type C Disease

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Niemann-Pick type C (NPC) disease is a fatal neurodegenerative disorder characterized by widespread intracellular accumulation of unesterified cholesterol and glycosphingolipids (GSLs). While there is no cure for NPC disease, there have been recent advances in the treatment of this disorder utilizing the compound 2-hydroxypropyl-beta-cyclodextrin (HPBCD). We and others have shown significant disease amelioration by administration of HPBCD to NPC mice. There are, however, three forms (alpha, beta, and gamma) and many different derivatives of cyclodextrins (CDs), raising the question of whether a different form or derivative would more be more efficacious in disease amelioration. Additionally, as the mechanism of action of CD is unresolved, treatment efficacy of different CDs may shed light on this very important question. To test which CDs were most efficient at reducing storage of cholesterol and gangliosides in brain, we performed two week in vivo experiments during which Npc1⁻/⁻ and +/+ mice were administered subcutaneous injections every other day of one of ten different CDs. Treatment was begun at postnatal day 7 (P7) and continued to P21. Mice were then sacrificed at P22 and immunohistochemical (IHC) and biochemical analyses were performed on tissues to evaluate efficacy of treatment.

We studied ten different CDs: hydroxypropyl-alpha-CD (HPACD), hydroxypropyl-beta-CD (HPBCD; purchased from Sigma-Aldrich), hydroxypropyl-gamma-CD (HPGCD), methyl-beta-CD (MBCD), sulfobutylether-alpha-CD (SBEACD), sulfobutylether-beta-CD (SBEBCD), sulfobutylether-gamma-CD (SBEGCD), Trappsol, Kleptose HPB, and Kleptose HP. Based on matched areas of neocortex, analysis of unesterified cholesterol (visualized by filipin labeling) and GSLs (GM2 and GM3 gangliosides visualized by IHC) revealed that the form and derivative of CD plays a key role in treatment efficacy. For example, neither HPACD nor SBEACD showed detectable storage amelioration, while administration of beta and gamma-CDs led to reduction of both cholesterol and GSL accumulation. Furthermore, the hydroxypropyl-beta and gamma CD derivatives were more effective at reducing ganglioside storage than were the sulfobutylether-beta and gamma CD derivatives. In terms of cholesterol, SBEBCD was least effective at ameliorating cholesterol storage when compared to HPBCD, HPGCD, and SBEGCD. Trappsol, Kleptose HPB, and Kleptose HP, all of which are FDA approved HPBCDs, showed reduction of cholesterol and GSL storage similar to the HPBCD purchased from Sigma-Aldrich. Biochemical analysis of GSLs corroborated these findings. Overall, CD treatment efficacy from greatest to least disease amelioration is as follows: HPBCD, Trappsol, Kleptose HPB, Kleptose HP > HPGCD, MBCD > SBEGCD > SBEBCD >> HPACD, SBEACD. These results confirm that the most effective CD for treatment of NPC disease is HPBCD, which is also known to have a good safety profile. A clinical trial, which is proposed to begin in late 2012 at National Institutes of Health, will utilize Kleptose HPB for intracerebroventricular administration to NPC patients. To further address the unresolved question of how CD mediates NPC disease amelioration, we are collaboratively studying the in vitro binding capabilities of several different CDs to various storage metabolites and other substrates important in NPC disease.
NPC-509: Establishment of a novel biomarker for the primary diagnosis and monitoring of Niemann-Pick Type C disease

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Biomarkers play an essential role in the primary diagnosis and the monitoring of chronic diseases, especially when there are treatment options available. However, for most lysosomal storage disorders – diseases that involve the dysfunction and early degradation of lysosomal enzymes with concurrent accumulation of the subsequently non-degraded compound – reliable biomarkers are still lacking.

By conducting a number of international, prospective, multi-center clinical trials – BioNPC, BioGaucher, BioHunt, BioKrabbe, BioMorquio, BioPompe, BioMLD and BioMaroteaux (for details see [www.clinicaltrials.gov](http://www.clinicaltrials.gov)) – we aim to obtain further insights in biomarkers for diagnosing and monitoring the individual lysosomal storage disorders.

Though these clinical trials are currently ongoing, we will report preliminary data on the recently identified biomarker NPC-509 for Niemann-Pick Type C disease (NPC). This biomarker was identified in a first screen using HPLC-MS/MS of blood plasma samples of a panel of 10 well characterized NPC patients and 10 age- and sex-matched healthy subjects. Following the first screen approximately 250 patients and controls have been analyzed for the new marker NPC-509. After demonstrating specificity and sensitivity of the new marker, we started to analyze the molecular weight and structure of the marker, especially for the synthesis of an internal standard for MS/MS-tandem analysis. Remarkably, this biomarker is neither elevated in other lysosomal storage diseases nor in NPC carriers nor healthy individuals and further allows for differentiation of patients suffering from NPC and NP A/B.

We report diagnostic sensitivity and specificity and first follow-up data. Importantly, the marker can be analyzed in plasma using the dry-blood-spot format which facilitates the logistical effort of the transport of the patient samples.

Summarizing, we detected a novel biomarker for NPC and will continue our clinical trial BioNPC, to further affirm the sensitivity and specificity of NPC509 – especially in the early manifestation of the disease - and ascertain a correlation between the burden of the disease and NPC509. Ultimately, by making a new biomarker available, we aspire to make diagnostic and monitoring procedures more feasible for physicians and patients alike.
Altered Energy Metabolism in Niemann-Pick Type C1-deficient Murine Brain

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The brain has one of the highest basal metabolic rates in the human body. Brain metabolism accounts for around 20% of the body’s basal oxygen consumption, in line with its heavy reliance on mitochondrial oxidative phosphorylation to generate the energy required for upkeep of membrane potential and synaptic activity. Most neurodegenerative diseases are associated with mitochondrial dysfunction, and most mitochondrial defects manifest in the brain. In Niemann-Pick Type C (NPC) disease, impaired lipid trafficking and cholesterol homeostatic responses also affect mitochondria, leading to increased mitochondrial membrane cholesterol, and, paradoxically, to decreased neurosteroid and oxysterol synthesis. Increased oxidative stress markers in NPC patients and in animal and cell models further suggest a role for mitochondrial dysfunction in NPC disease pathology. Changes in mitochondrial function could affect overall brain energy and neurotransmitter metabolism. To investigate energy metabolism in NPC disease, we performed ¹H-NMR spectrometric metabolomic analyses of different brain regions from NPC1-deficient mice at three stages of the disease. Metabolic profiling was complemented with gene and protein expression analysis of key enzymes and regulators of energy and neurotransmitter metabolism. We found significant differences in the metabolic and gene expression profiles of NPC1-deficient murine cerebellum, compared to age-matched wildtype cerebellum. Some of these changes were observed presymptomatically, and before widespread cell death in the cerebellum. Our findings indicate that energy metabolism is altered in NPC1-deficiency and could affect neurotransmitter balance.
Chronic Administration of 2-Hydroxypropyl beta Cyclodextrin Directly into the CNS of NPC Mice Greatly Delays Neurodegeneration

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2-hydroxypropyl beta cyclodextrin (2HPBCD) has been shown to reverse the cholesterol transport defect in NPC disease and allow the trapped cholesterol within the late endosome/lysosome (LE/LY) to be released into the cytosolic compartment where it is then normally metabolized. Systemic administration of 2HPBCD to Npc1<sup>−/−</sup> mice starting at 7 days of age and repeated weekly ameliorates the disease in most of the peripheral organs such as the liver and spleen and significantly extends lifespan. However, neurodegeneration, while delayed, is not able to be completely prevented due to the inability of 2HPBCD to effectively penetrate the blood brain barrier (BBB). We have previously demonstrated that when the BBB is bypassed and 2HPBCD is delivered as a continuous 4 week infusion directly into the CNS, in addition to systemic administration of 2HPBCD starting at 7 days of age, neurodegeneration during this treatment period is able to be completely prevented. However, long term treatment with 2HPBCD has yet to be explored.

In a series of experiments, therefore, we explored the effects of chronic long term infusion of 2HPBCD into the CNS of NPC mice. Npc1<sup>+/+</sup> and Npc1<sup>−/−</sup> mice were treated with subcutaneous (SQ) 2HPBCD starting at 7 days of age along with continuous intracerebral ventricular (ICV) administration of either artificial cerebral spinal fluid (aCSF) or 2HPBCD via osmotic pump starting at ~ 21-25 days of age. The mice were studied at 100 days of age at which time brain was taken for histology and brain and liver cholesterol levels were measured. As expected, with chronic systemic treatment of 2HPBCD, the liver cholesterol content in Npc1<sup>−/−</sup> animals was dramatically reduced. In Npc1<sup>−/−</sup> animals that received ICV infusion of 2HPBCD, brain cholesterol levels improved toward normal levels. On histological examination of the brain, Npc1<sup>−/−</sup> mice that received aCSF through the ICV pump had significant lipid accumulation within the neurons as well as complete loss of Purkinje cells. In contrast, animals that received 2HPBCD through the ICV pump had nearly complete absence of intraneuronal lipid accumulation and the Purkinje cells were well preserved even at this advanced age. mRNA levels of genes involved with neuroinflammation were also improved in Npc1<sup>−/−</sup> mice treated with continuous ICV infusion of 2HPBCD.

In humans, NPC disease is typically diagnosed once children begin to demonstrate symptoms, which typically occurs at a later age. In the NPC mouse model, it has been observed that systemic SQ administration of 2HPBCD is most effective at extending lifespan when it is initiated at a very young age (P7) when the mice are asymptomatic. This beneficial effect on lifespan is diminished when 2HPBCD is initiated at later ages and completely lost when Npc1<sup>−/−</sup> mice are treated with 2HPBCD starting at 49 days of age when symptoms become evident. While the peripheral organs improve with 2HPBCD treatment, these mice still succumb to neurodegeneration due to the inability of 2HPBCD to effectively cross the BBB. Therefore, in a second set of experiments, to more closely mimic the human disease course in which treatment is usually not initiated until an older age when symptoms become apparent, we set out to answer whether initiating treatment with both weekly systemic SC administration and continuous ICV delivery of 2HPBCD in Npc1<sup>−/−</sup> mice starting at 49 days of age would improve survival. These experiments are currently still ongoing and the results will be reported at the conference.
Molecular mechanism of $\beta$-cyclodextrin mediated cholesterol extraction from biomembranes: key to understand its palliative effect in Niemann-Pick type C disease

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Niemann-Pick type C (NPC) disease is characterized by the accumulation of unesterified cholesterol (UC) within the late endosomal/lysosomal compartment of all cells in the body, due to a defect in cholesterol trafficking, which leads to cellular dysfunction and, ultimately, cell death. Cyclodextrins are able to overcome this cholesterol trafficking defect and allow the abnormally sequestered UC within the late endosome/lysosome (LE/LY) to move into the cytosolic compartment where it is then normally metabolized.

Despite the considerable number of studies supporting the ability of this molecule to regulate the membrane cholesterol content in vivo and in situ, the molecular aspects are not yet completely established. To provide such a detailed view, we present the results of a systematic series of molecular dynamics (MD) simulations of the interaction between $\beta$-cyclodextrin and cholesterol/lipid membrane models. We show that cholesterol can be extracted efficiently upon adsorption of CD dimers at the membrane/water interface. However, extraction is only observed to occur spontaneously in membranes with high cholesterol levels. Free energy calculations reveal the presence of a kinetic barrier for cholesterol extraction in the case of low cholesterol content, resulting from the shielding of the cholesterol molecules by adjacent lipid head groups. Cholesterol uptake is facilitated in case of (poly)unsaturated lipid membranes, which increase the free energy of the membrane bound state of cholesterol. Comparing typical lipid/cholesterol compositions of liquid-disordered ($L_d$) and liquid-order ($L_o$) domains, we furthermore show that cholesterol is preferentially extracted from the disordered regions, in line with recent experimental data.

The results of this investigation constitute a solid theoretical model for $\beta$-cyclodextrin mediated cholesterol depletion from biomembranes that may aid in interpreting experimental data. We hope it will also contribute to the design of more effective cyclodextrin derivatives for medical treatment of lipid metabolism pathologies, like for example in the treatment of Niemann-Pick type C disease.
Abstract NPC Conference

**In vitro modeling of inherited neurodegenerative cholesterol storage disorder using patient-specific induced pluripotent stem cells (hiPSCs)**

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Patient-specific induced pluripotent stem cells (hiPSCs) derived from somatic tissues provide a unique tool to study human diseases *in vitro* and to develop new therapeutic strategies. These cells also have potential to generate unlimited quantities of various cell types for autologous transplantation and therefore may one day be used to treat patients suffering from various degenerative diseases. However the use of hiPSCs in the context of genetically inherited diseases requires the correction of disease-causing mutations without leaving large residual sequences in the targeted genome.

Niemann-Pick disease type C (NPC1) is an inherited, progressive neurodegenerative disorder caused by mutations in the NPC1 or NPC2 gene. Mutations in these genes result in an accumulation of unesterified cholesterol in the late endosomes / lysosomes (LE/L) and an impairment of the cholesterol export from the LE/L to the endoplasmic reticulum (ER). The first clinical symptoms are often hepatosplenomegaly and cholestasis. The course of the disease is dramatic and subsequently leads to the death of the patient.

In the presented study we have generated hiPSCs of patients suffering from NPC1 disease. Derived human NPC1 iPSCs are free of reprogramming factors and have been characterized for pluripotency and their differentiation capacity. Using different biochemical approaches we were able to evaluate a disease-related phenotype in a variety of different cell types including cells of the neuronal and hepatic lineage. Currently we are investigating the effect of engineered transcription activator-like effector nucleases (TALENs) mediated gene repair in generated patient-specific NPC1 iPSCs.
Testing the effect of drugs on arrays of NPC1 mutations

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Abstract

95% of Niemann–Pick disease type C (NPC) disease is caused by mutations in a transmembrane protein, NPC1, that block efflux of cholesterol from late endosomes and lysosomes. Previous studies have found that histone deacetylase inhibitors (such as LBH589) are able to correct the NPC1 phenotype in human fibroblast cells with an NPC1 I1061T mutation. NPC1I1061T is the most common mutation observed in NPC1 patients. However, there are more than 100 different mutations in NPC1 patients. The effects of drugs on NPC1 mutations other than NPC1 I1061T are still unclear. In order to examine the effectiveness of histone deacetylase inhibitor (HDACi) treatment on hundreds of different NPC1 mutations simultaneously, a 384-well plate screen has been designed. The NPC1-null human osteosarcoma cell line (U2OS_shNPC1) is used in the screen. U2OS_shNPC1 cells are transfected with mutated NPC1 and GFP coexpression vector (pMIEG3-NPC1) by reverse transfection. The mutated NPC1 protein is expressed in GFP positive cells. A plating protocol was designed to add all products in 384 well plates robotically. Using this system, we can test the effects of drug treatments on many different NPC1 mutations. The first application will be to determine which NPC1 mutations can be corrected by HDACi’s. This should help in evaluating whether or not HDACi treatment is suitable for patients carrying these mutations. We have tested LBH589 and SAHA on over 50 different NPC1 mutations. HDAC’s (LBH589 and SAHA) can be used to treat Niemann–Pick disease type C resulting from many different NPC1 mutations.
Testing Histone Deacetylase Inhibitors as NPC Therapeutics

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In collaboration with the laboratories of Drs. Helquist and Wiest (Notre Dame), we described the use of histone deacetylase inhibitors (HDACi) to reduce the cholesterol accumulation in human fibroblasts expressing mutant forms of the NPC1 protein (1). The initial studies were carried out using two human lines. One of these was homozygous for the \textit{NPC1}^{I1061T} mutation, and the other was heterozygous with one \textit{NPC1}^{I1061T} allele. The HDACi treatment increased the expression of the NPC1 protein, and this increased protein expression may have been a major reason for the reversal of the cholesterol accumulation. We have extended the testing of the HDACi to several other mutations. First, we tested several patient fibroblast cell lines provided by Dr. Forbes Porter (NIH). Second, in collaboration with William Balch’s laboratory (Scripps), we have created a human cell line that does not express endogenous NPC1 but can be efficiently transfected with cDNAs encoding various known mutations in NPC1. We are testing the ability of the HDACi to reverse the cholesterol accumulation in cells expressing these various mutations. This should help to identify mutations that are susceptible to rescue by HDACi – at least at the cellular level.

We have found that several of the human patient cell lines with different point mutations in NPC1 have their cholesterol storage reduced greatly upon treatment with HDACi. Additionally, many of the NPC1 point mutations expressed in our engineered cell line show significant correction of the cholesterol accumulation only when treated with HDACi. These data suggest that HDACi treatment may be beneficial for a large fraction of NPC1 mutations. We have also found that treatment of NPC1 mutant cells with HDACi increases the amount of NPC1 protein that is detectable in endocytic/lysosomal compartments.

Cholesterol transport by NPC2 and the therapeutic use of cyclodextrin polymers in NPC cells
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Niemann-Pick type C (NPC) disease is characterized by the accumulation of cholesterol and other lipids in the late endo/lysosomal compartment, and is caused by defects in either of two genes that encode for the proteins NPC1 and NPC2. NPC2 is a 16kDa soluble lysosomal protein that binds cholesterol. To determine the role of NPC2 in lysosomal cholesterol trafficking, we developed a set of novel fluorescence-based assays to monitor the kinetics of cholesterol transfer from NPC2 to model membranes, from membranes to NPC2, and transfer of the cholesterol analog, dehydroergosterol (DHE), between membranes. Results show that NPC2 rapidly transports cholesterol to/from phospholipid vesicles via direct interaction with the membranes, and rates are greatly enhanced by the unique lysosomal phospholipid lyso-bisphosphatidic acid (LBPA). Site-directed mutagenesis, assessed using the sterol transfer assays and studies of npc2−/− fibroblasts, has suggested that at least two membrane interacting domains may be present on the surface of NPC2, and be necessary for its cholesterol transport properties. A turbidity assay was used to test the possibility that NPC2 is able to interact with greater than one membrane simultaneously; a dose-dependent increase in turbidity upon addition of NPC2 to unilamellar vesicles indicates that NPC2 can cause vesicle-vesicle interaction, supporting the hypothesis that the surface of NPC2 contains two membrane interaction domains. We have also examined the cholesterol transport properties of cyclodextrins (CD), shown recently to have therapeutic potential when administered to NPC1 and NPC2 deficient mice. Results show that CD enhances intermembrane sterol transfer rates in a manner similar to NPC2, however no effects of LBPA were found for CD, in contrast to NPC2. Since the rapid elimination of monomeric CD from circulation reduces the therapeutic efficacy of these compounds, however, a novel group of CD polymers has been developed by the Thompson laboratory (Purdue) to address this problem. Addition of these compounds to npc2−/− fibroblasts appears to result in rapid elimination of intracellular cholesterol. Effectiveness at the cellular level indicates that these compounds, which should have longer circulation times in vivo, may prove useful in therapeutic application to NPC disease.
Translation of disease modifiers from yeast to mouse models of Niemann-Pick type C disease

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Given the extensive clinical variation in NPC disease and the rare occurrence of genotype-phenotype correlations for mutations in NPC1 or NPC2, it is likely that there are genes other than NPC1 or NPC2 modifying the onset and progression of NPC disease. It is our premise that these genes represent therapeutic targets to treat NPC disease. We have established an “exacerbate-reverse” approach to identify genetic modifiers of NPC disease using unbiased, genome-wide analyses of the yeast model of NPC disease (Munkacsi et al., J. Biol. Chem., 2011) and identified 12 pathways that exacerbate lethality in yeast that can potentially be reversed in human cells as a novel therapeutic strategy. One pathway is that of histone acetylation homeostasis; deletion of a histone acetylase gene conferred inviability in the yeast model of NPC disease. In proof-of-principle experiments that validated our exacerbate-reverse approach, we used a clinically approved HDAC inhibitor (suberoylanilide hydroxamic acid, SAHA, Vorinostat, Zolinza®) to treat human NPC patient fibroblasts and reversed the major diagnostic criteria of NP-C disease: lysosomal accumulation of both cholesterol and sphingolipids and defective esterification of LDL-derived cholesterol. To translate these in vitro results in fibroblasts to in vivo results in animal models, we first determined that SAHA crosses the blood-brain barrier in P21 mice; concentrations in plasma and brain were obtained at 60 μM and 3 μM, respectively. This treatment regime resulted in micromolar concentrations in the plasma and brain that were previously used to rescue lipid accumulation in NPC patient fibroblasts. Experiments are now in progress to determine if SAHA can ameliorate cholesterol and sphingolipid accumulation in the liver and brain at pediatric, adolescent and adult stages of the Npc1<sup>l<sup>1</sup>mt<sup>164</sup> </sup> mouse. A second modifier pathway we identified using the exacerbate-reverse approach was the pathway regulating homeostasis of phosphatidic acid (PA) and diacylglycerol (DAG); the deletion of Phosphatidic Acid Hydrolase (encoded by PAH1 in yeast and LPIN1, LPIN2 and LPIN3 in mammals) markedly exacerbated lethality of the yeast model. PAH1 catalyzes the conversion of PA to DAG, thus the therapeutic strategy that arises from this finding lies in inhibiting the synthesis of PA as accomplished by pharmacologically inhibiting either diacylglycerol kinase or phospholipase D. The exacerbate-reverse method described here has produced unexpected therapeutic strategies that are currently under development.
TITLE: Use of induced pluripotent stem cells to model selective neuronal failure in Niemann Pick type C1

AUTHORS: M. Paulina Ordonez, MD, and Lawrence S.B. Goldstein, PhD

INSTITUTION: University of California San Diego

ABSTRACT:

Niemann-Pick type C1 (NPC1) is a progressive and uniformly fatal inherited pediatric dementia, characterized by defects in intracellular cholesterol trafficking. NPC1 is caused by loss of function of the endosomal-lysosomal membrane transporter NPC1. Disruptions in this transport system result in the accumulation of cholesterol and glycolipids in the lysosomal compartment, triggering progressive cerebellar, hippocampal, and cortical neurodegeneration. NPC1 has intriguing similarities with Alzheimer’s disease, suggesting a common pathogenic mechanism. Previous research in our lab utilizing genetically engineered NPC1 knockdown (KD) human embryonic stem cells (hESC) suggests that disrupted turnover of mitochondria by autophagy may be a major factor causing selective neuronal failure. Utilizing retroviral reprogramming methods we have generated and characterized a set of induced pluripotent stem cells (iPSC) from NPC1 null and severe hypomorph patient fibroblasts. We efficiently induce neuronal differentiation of control and NPC1 hIPSCs using specific growth factor combinations and enrichment of neuronal populations by fluorescence activated cell sorting (FACS). Our main goal is to use patient specific NPC1 neurons, and hESC-derived NPC1 knockdown neurons previously generated in our lab to probe alternative mechanisms of disease that will lead to effective therapeutic approaches for NPC1 and related disorders. Specifically, we are testing the hypothesis that NPC1 mimics a state of cholesterol starvation, inducing excessive activation of autophagy as an alternative route to release cholesterol from the lysosome, and that imbalanced autophagy is a major contributor to selective neuronal failure, triggering downstream pathologies typical of disease progression including synaptic defects. The use of hIPSCs to study human neurodegenerative disease is in the early stages of development and validation of the use of these cells and their derivatives to model human disease is crucial. Parallel analysis of neurons derived from NPC1 KD hESCs and patient specific hIPSCs is a powerful approach that will begin to assess the role of genetic heterogeneity in generating neuronal phenotypes, and therefore will help cross validate the use of hIPSCs for the study of NPC1. Additionally, our approach establishes a cell-based platform for the high-throughput screening of potential therapeutic compounds for NPC1 and related neurodegenerative diseases.
Oxysterol Biomarkers for Niemann-Pick C1 Disease

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Niemann-Pick type C1 (NPC1) disease is a rare, progressively fatal neurodegenerative disease for which there are no FDA-approved therapies. A barrier to the development of effective treatments for NPC1 disease is the lack of biochemical outcome measures to evaluate efficacy of therapy in clinical trials. The availability of blood and tissue samples obtained from a carefully phenotyped cohort of human NPC1 subjects enrolled an NIH-sponsored natural history study has provided a unique opportunity for discovery of NPC1 disease biomarkers. Our work with this cohort resulted in identification of two cholesterol oxidation products – cholestane-3β,5α,6β-triol (3β,5α,6β-triol) and 7-ketocholesterol – that are elevated nearly 10-fold in the plasma of NPC1 subjects. These cholesterol oxidation products (“oxysterols”) have led to development of a rapid, highly-sensitive and specific, non-invasive blood test for NPC1 disease that is being implemented in clinical laboratories in North America, South America and Europe. We anticipate that the oxysterol test will replace filipin staining as the diagnostic standard for this disorder. The utility of these oxysterol biomarkers as biochemical outcome measures for monitoring response to therapy is being examined in animal models, and are expected to serve as guides for dose-escalation studies in the planned Phase I cyclodextrin trial. We have further extended this technology to development of prototype, high-throughput LC-MS/MS assay for detection of 3β,5α,6β-triol in dried blood spots from the newborn screening cards. Early diagnosis of NPC1 disease would facilitate treatment in presymptomatic subjects – individuals most likely to benefit from medical intervention – and would be expected to significantly delay disease onset and prolong survival.
Unfinished journey: Niemann-Pick disease, type C in its seventh decade.

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Niemann-Pick disease, type C (NPC) was born in confusion at the time of its first clear description in 1958, when it was erroneously grouped with the primary acid sphingomyelinase deficiencies, subsequently categorized as Niemann-Pick diseases, types A and B. This talk traces the history of the disease in the context of rapidly changing approaches to science (from hypothesis-driven experimentation to industrial ‘brute-force’ screening strategies) and advocacy (from parents as caregivers to parental entrepreneurs driving research), the latter being a major stimulus to a vastly expanded research enterprise in NPC. Two causative genes have been cloned and the function of their gene products is beginning to be understood. One disease-modifying therapy has been approved in many countries outside the US, and there are now almost as many drug candidates for clinical trials as there are suitable trial participants. No current proposed therapy is curative, but combination therapies have the potential to improve the duration and quality of life in people with NPC. The identification of elevated oxysterol species promises to permit early diagnosis (if the test is applied appropriately in screening protocols); previous and current studies of disease intervention have been and are hampered by late diagnosis and a substantial burden of irreversible neurologic disease at presentation. The effects of aggressive supportive care have not been studied systematically, but clinical observation and experience in analogous disorders argues that better symptomatic therapy has improved outcomes even before disease-modifying therapies were introduced. Perhaps most exciting, rapid advances in DNA sequencing hold the promise of disease prevention, a strategy whose effectiveness has been demonstrated dramatically in another lysosomal storage disorder, Tay-Sachs disease, whose incidence has fallen over 95% in its target population since widespread screening was implemented.
Psychology and psychiatry: needs and fears of individuals with Niemann-Pick Type C (NP-C)

PD Dr. med. Hans-H. Kluenemann, Lisa Peintinger, Clinic and Policlinic for Psychiatry and Psychotherapy, Regensburg, GER

Following Wijburg et al. (2011), who developed the “Suspicion Index” to rank visceral, neurological and psychiatric symptoms, we want to generate another prognostic tool that further helps to diagnose NP-C more precisely and at the earliest stage possible. Our aim is to raise the clinician awareness of this recent disease, so misdiagnoses can be prevented. Furthermore we want to focus on the psychological aspects of this illness and to explore possibilities, given to support NPC-patients and their families. Could psychotherapy be helpful in the management of this genetic disorder or would it be necessary to have more group therapy and information for people who are concerned with this? Focus is on needs and fears of affected families.

When we look back, there had been several studies doing research on successful treatment or therapy on Niemann-Pick disease type C. Pérez-Poyato & Pineda (2001) summarized recent treatments that have been explored to cure NP-C. Despite there is still a lack of knowledge in psychological care.

We reviewed and analyzed 25 cases of NP-C patients in Germany, Austria and Switzerland to identify specific neuropsychological as well as psychiatric signs, e.g. psychosis, paranoid delusions, hallucinations, depression, etc. (Weber & Kluenemann, 2011), of NP-C. Neuropsychological testing examines different areas in the brain, which are responsible for cognitive functions like attention, concentration, memory, language, motor skills, etc. Thereby, scores of the tests show declines of cognitive functions of the patients suffering on NP-C. After a retrospective analysis of clinical records, we plan to interview patients and their family members about their emotional states, needs and anxieties. These qualitative surveys bring additional information helping to create an appropriate support as well as to extent the knowledge about individuals confronted with a fatal disease.

The knowledge about the psychiatric phenotype could improve the understanding of Morbus Niemann-Pick type C. Psychological aspects would help to explain the behavior of individuals with NP-C that sometimes seems intransparent for their parents or siblings. Further research would describe the psychological state of their daily caregivers and how patients and caregivers deal with the difficulties of this illness.

Cholesterol export from lysosomes by NPC1 and NPC2 proteins
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NPC1 protein is needed for cellular utilization of low density lipoprotein-derived cholesterol that has been delivered to lysosomes. The protein has 13 transmembrane domains, three large lumenal domains, and a cytoplasmic tail. NPC1’s lumenally oriented, N-terminal domain binds cholesterol and has been proposed to receive cholesterol from NPC2 protein as part of the process by which cholesterol is exported from lysosomes into the cytosol. Using surface plasmon resonance and affinity chromatography, we have shown that the second lumenal domain of NPC1 binds directly to NPC2 protein (1). Interaction of NPC2 with NPC1 lumenal domain 2 is only detected at acidic pH and requires the presence of cholesterol on NPC2. Disease-causing mutations in NPC1 decrease NPC2 binding, suggesting that NPC2 binding is necessary for NPC1 function in humans. These data support a model in which NPC1 domain 2 holds NPC2 in position to facilitate directional cholesterol transfer from NPC2 onto NPC1 protein for export from lysosomes. Mutagenesis of NPC2 protein has identified residues that are needed for interaction of NPC2 with NPC1 protein; our model is that the second lumenal domain of NPC1 binds to the “back side” of NPC2 to permit the “front” of the protein to transfer cholesterol to NPC1’s N-terminal domain.

Why do cells need NPC1 and NPC2 for cholesterol egress from lysosomes? The inner membrane of lysosomes is lined with a glycocalyx that is thought to protect the membrane proteins and lipid bilayer from lysosomal proteases and lipases. This layer would theoretically block spontaneous cholesterol insertion into the membrane. We have carried out experiments to alter the structure of the glycocalyx and find that cells with a less elaborate glycocalyx are less dependent upon NPC1 protein for cholesterol export: these cells accumulate less cholesterol in lysosomes when NPC1 function is blocked. These data support the glycocalyx model and suggest that modification of lysosomal glycoprotein structure might be beneficial for patients with NPC1 disease. Experiments are in progress to dissect further, the mechanism of cholesterol transport out of lysosomes.

Cell-specific pathologic changes in retinae of NPC1-deficient mice

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Cells in the central nervous system show remarkable differences in their vulnerability to the absence of NPC1 protein. Cerebellar Purkinje cells degenerate rapidly, whereas neurons of other brain regions or astroglial cells appear relatively unaffected. Based on our previous studies (Mauch et al., 2001; Göritz et al., 2005; Nieweg et al., 2009), we postulated that retinal ganglion cells (RGCs) depend on the endocytotic import of glia-derived cholesterol and that they are affected by the lack of NPC1. Our first analysis of retinae from NPC1-deficient mice revealed an accumulation of autophagosomes in the ganglion cell layer at two months of age and pathologic changes in other retinal cells, notably photoreceptors (Claudepierre et al., 2010). To explore the link between autophagy and NPC1 in ganglion cells, we have now established a pharmacologic assay based on serum-free primary cultures of highly purified RGCs. Treatment of cells with U18666A induced an accumulation of unesterified cholesterol in the endosomal-lysosomal system, enhanced the level of lysobisphosphatidic acid, a late-endosome marker, and increased the number of LC3-positive particles. Notably, these effects occurred only in the presence of glia-conditioned medium suggesting a requirement for endocytotic uptake of cholesterol. We are now using our assay to test the effects of candidate drugs like cyclodextrin on U186663-induced modifications in RGCs. In parallel, we have started to explore the integrity of the blood-retina barrier. These experiments were prompted by our recent electron microscopic observation that vascular endothelial cells in retinae of NPC1-deficient mice show signs of autophagy. Using tail-vein injections of fluorescently-labeled beads, we obtained first evidence for a breakdown of the intraretinal barrier in 10-weeks-old NPC1-deficient mice. Finally, we examine in-depth the retinal pigment epithelium, which appears particularly sensitive to the lack of NPC1. In 10-weeks-old mice, RPE cells lacking NPC1 show dysmorphic mitochondria as well as accumulation of lipofuscin and lipid droplets.

Together, our study reveals a whole spectrum of cellular reactions to NPC1 deficiency in the retina, notably in the ganglion cell and pigment epithelium layer, and suggests a - previously unnoticed - vulnerability of the blood-retina barrier. We aim now to define the molecular basis of the cell-specific pathologic changes. Our results may help to identify and validate new drug targets and to design efficient tests for the treatment of NPC disease.
Development of a hydroxypropyl-β-cyclodextrin therapeutic trial for Niemann-Pick disease, type C1

Forbes D. Porter1 and the TRND Team2

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Niemann-Pick Disease, type C1 (NPC1) is a rare, autosomal recessive, neurodegenerative disorder due to mutation of the NPC1 gene. Lack of NPC1 function results in endolysosomal accumulation of unesterified cholesterol and lipids. Although the signs and symptoms observed in individual patients vary, the NPC1 neurological phenotype is characterized by progressive cerebellar dysfunction and cognitive impairment. While miglustat, a glycosphingolipids synthesis inhibitor, has been approved for use in Europe and other countries, there are no FDA approved therapies for NPC1.

As part of an ongoing Natural History study of NPC1, we have evaluated clinical aspects of over sixty NPC1 patients. This work has laid the foundation for the development of biomarkers that will facilitate both diagnosis and provide tools to facilitate the development of a clinically effective therapy for NPC1 patients. Hypothesis-based and de novo discovery approaches have been used to characterize potential protein, lipid and sterol biomarkers in both serum and cerebral spinal fluid from NPC1 patients and to correlate these findings with disease status. A number of these biomarkers including oxysterols, calbindin D, fatty acid binding protein 3, macrophage inhibitory protein 1 alpha, cathepsin D, superoxide dismutase, and galectin-3 give insight into various aspects of the NPC1 pathological cascade and will provide tools to guide rational development of a therapeutic trial.

Hydroxypropyl-β-cyclodextrin (HP-β-CD) has been shown to have remarkable therapeutic efficacy in both the mouse and cat models of NPC1. The degree of efficacy in animal models exceeds that of other candidate drugs. HP-β-CD has previously been approved by the FDA as an excipient. In a collaboration coordinated by the new National Center for Advancing Translational Sciences (NCATS), a trans-NIH, intramural/extramural effort is underway to initiate a Phase I clinical trial of intracerebroventricular administration of HP-β-CD for NPC1. The plan being proposed to the FDA and NICHD IRB will involve cohort dose escalation to establish a safe and biochemically effective dose in a systematic and scientifically rigorous manner. This study will determine the safety and pharmacokinetics of HP-β-CD. In addition, pharmacodynamic responses of biomarkers will be used to establish biochemical and pathological efficacy. In anticipation of establishing a safe and biochemically effective dose in our Phase I study, we are planning a multicenter, likely international, trial to determine the clinical efficacy of HP-β-CD.
Comparing the Molecular Mechanisms of Novel Therapies for NPC Disease

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Niemann Pick type C disease is a genetic disorder in which lipids (unesterified cholesterol, sphingomyelin, and glycolipids) accumulate in the late endosomes/lysosomes of cells throughout the body, resulting in liver, lung and neurologic symptoms. Our work has identified two agents that act in the Npc1−/− mouse model to enhance Purkinje cell survival, alter cholesterol dynamics in the central nervous system, reduce inflammation, and prolong lifespan: the LXR agonist T0901317 (oral dosing at 50 mg/kg body weight) and 2-hydroxypropyl-β-cyclodextrin (2HPBCD, provided as single or repeated subcutaneous injections at 4000mg/kg body weight)1,2.

Despite these beneficial effects, each drug has limitations: 2HPBCD fails to affect the progression of lung disease in the Npc1−/− mouse model3,4 and T0901317 is associated with hepatic steatosis. Thus we have also begun to evaluate combination therapies (LXR agonist + 2HPBCD) with a new focus on pulmonary function. The LXR agonist, T0901317, elicits changes in both lung and CNS suggesting that it can penetrate these organ systems to produce an additive effect on survival in the Npc1 mouse. Anna Taylor will report on the results of studies utilizing dual LXR/CYCLO therapy.

As reported at previous NPC Scientific Conferences and recently published5, a high-throughput screening effort spearheaded by the Wiest and Helquist labs at Notre Dame (Chemistry Core) and the Maxfield group (human NPC fibroblast filipin-based screening strategy) identified HDAC inhibitors as novel compounds that reduce lysosomal cholesterol accumulation in human NPC fibroblasts. Similar findings using cultured human fibroblasts were also reported by the Sturley group6. Our lab continues in collaborative studies on the most potent of the HDAC inhibitors, panabinostat=LBH589. The therapeutic efficacy of LBH589 was tested in the Npc1−/− mouse (subcutaneous injection at 10 mg/kg body weight), and found to affect cholesterol homeostasis in CNS, liver, lung, and carcass; reduce inflammation; and improve liver function – but did not impact lifespan in mice lacking NPC1. In addition, the changes in sterol homeostasis were also observed in wildtype mice, so do not appear to rely on the cellular redistribution of cholesterol by egress from lysosomes in this mouse model. At least three primary differences can be identified to account for the alternative results observed in cultured human fibroblasts and the Npc1−/− mouse model: species-specific action of LBH589; HDAC inhibitor function dependent on cellular proliferation status; differential effects depending on Npc1 mutation status (mouse contains no NPC1 protein, some fibroblast lines express low and/or misfolded NPC proteins). We are systematically addressing these three differences to determine why/how LBH589 acts in human fibroblasts but does not improve disease progression in this mouse model, and will present our results.

Niemann-Pick Type C (NPC) is a neurodegenerative disease caused by the defective function of either NPC1 or NPC2 protein leading to the accumulation of unesterified cholesterol and other lipids at the late endosomal/lysosomal (LE/L) compartments. Over ~244 disease-causing mutations in NPC1 have been reported in the clinic and they include both missense and nonsense mutants. The most prevalent NPC1 mutant, I1061T is misfolded and shows a trafficking defect preceding delivery to the LE/L compartments resulting in a cholesterol imbalance in the cell. The trafficking and localization of the other NPC1 mutants is poorly understood. To date, we have generated a collection of plasmids that each harbor one of ~120 distinct NPC1 mutations that are representative of disease causing, loss-of-function mutants found in the different domains of NPC1. Each of these mutants have now been characterized in terms of their folding, trafficking and function in human NPC1-deficient (np1c/-) cells lines and correlated in many cases with NPC1 mutant phenotypes found primary cell lines obtained from NPC1 patients (Coriell Cell Repositories). We have used proteostasis regulators (PRs), small molecules that alter the folding environment of the cell, to restore the folding, trafficking and function of NPC1 mutants (Roth and Balch (2010) Curr. Opin. Cell Biol., 23:1; Balch et al. (2011) Cold Spring Harb. Perspect. Biol. 3 (2); Calamine B et al., (2012) Nat. Chem Biol., 8(2): 185-196). Application of PRs, which we now include many histone deacetylase inhibitors (HDACi), in an ongoing collaboration with Drs. Maxfield (Cornell), Runz (EMBL), Helquist (Notre Dame) and Wiest (Notre Dame) exhibit corrective responses reflecting the mutant phenotype which, in many cases, favors the trafficking of human NPC1 mutants into LE/L compartment and restoration of cholesterol homeostasis in NPC1-defective cells. By studying the impact of PRs/HDACi on a wide range of mutants spanning the entire domain organization of NPC1 we will be able to group the mutant phenotypes into classes that may be subject to global and/or personalized clinical correction of function that could provide benefit in achieving restoration of normal cholesterol homeostasis to a broad spectrum of NPC1 variants.
Combining Cyclodextrin and LXR Agonist Therapies as Treatment for Niemann Pick Type C Disease

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Proteins, NPC1 and NPC2, are required to traffic cellular cholesterol from the lysosome. Mutations in either gene lead to entrapment of cholesterol in the lysosome and cause Niemann Pick Type C disease (NPC), which presents as hepatic steatosis, splenomegaly, pulmonary dysfunction, and neurodegeneration resulting in premature death. As there are no approved pharmacological agents to extend the lifespan of patients with NPC, our lab has sought to identify compounds that increase the lifespan of the Npc1⁻/⁻ mouse. First, we found that an agonist for the oxysterol nuclear hormone receptor, Liver X Receptor (LXR), increases cholesterol efflux from brain, reduces neuroinflammation, and delays neuronal cell death, which leads to a modest but significant increase in lifespan. However, the LXR agonist used causes hepatic steatosis and splenomegaly, two symptoms already aggravated in NPC. Then in collaboration with the Dietschy group, we found that administration of 2-hydroxypropyl-β-cyclodextrin (HP-β-CD), a cholesterol-binding agent, elicits the release of trapped lysosomal cholesterol leading to a dramatic increase in lifespan. Unfortunately, HP-β-CD administration does not relieve the lung disease observed in the Npc1⁻/⁻ mouse and does not appear to reach the brain after the mature blood-brain-barrier has formed. This observation brought us back to the LXR agonist, which has been shown in other models to reduce pulmonary inflammation and can cross the mature blood-brain-barrier.

Hypothesis: Coadministration of HP-β-CD & an LXR agonist will have an additive effect on relieving NPC disease progression.

Research design and methods: One-week old wildtype and Npc1⁻/⁻ pups were injected weekly with either HP-β-CD (4000 mg/kg bw in Saline) or Saline. At 3 weeks of age, mice were weaned onto a powdered rodent diet with or without LXR agonist (20mg of T0901317/kg bw). The first cohort of mice underwent pulmonary function testing (Unrestrained Plethysmograph, Buxco) twice per week during their survival study. A second cohort of mice was euthanized at 7 weeks of age to harvest tissues for mRNA analysis by qPCR and for immunohistochemical evaluation of macrophages and cerebellar Purkinje cells.

Results: Npc1⁻/⁻ mice treated in combination with HP-β-CD & LXR agonist lived significantly longer than those treated with controls; however, there was no added benefit in survival over treatment with HP-β-CD alone. Regardless of treatment, Npc1⁻/⁻ mice had more difficult breathing in Pulmonary function tests than wildtype mice. mRNA analyses verified that both treatments worked as when compared to control mice, LXR agonist treated mice have increased LXR target genes and HP-β-CD treated Npc1⁻/⁻ mice have decreased SREBP2 target genes.

Conclusion: Dual treatment of HP-β-CD & LXR agonist in Npc1⁻/⁻ mice did not significantly increase lifespan over HP-β-CD treatment alone and did not significantly improve pulmonary function. Thus, providing the treatments in combination appears to have no added benefit and suggests that the two agents are working through a common mechanism.

References:

Regulation of Cholesterol Homeostasis with Spliceosome Inhibitor Herboxidiene, A Potentially Novel Lead for Niemann-Pick Type C Disease.

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Recent studies have shown that histone deacetylase (HDAC) inhibitors can correct cholesterol storage defects in human NPC1 mutant cells provide the potential basis for treatment options for NPC disease. Presumably, the HADC inhibitor, panobinostat, can correct cholesterol homeostasis through its ability to broadly upregulate gene expression. Herboxidiene, is a Streptomyces-derived polyketide with anti-tumor activity. Herboxidiene’s ability to upregulate gene expression is similar to trichostatin A, a known HDAC inhibitor. However, herboxidiene does not affect histone acetylation but has recently been shown interact with SP155 and inhibit pre-RNA splicing. Based on these findings, we sought to investigate herboxidiene’s potential to affect cholesterol levels in NPC cells. Efforts to produce significant quantities of herboxidiene from the fermentation of Streptomyces chromofuscus and its evaluation in NPC cells will be presented.
Synthesis and Characterization of Non-covalent β-Cyclodextrin Polymer Derivatives for Evaluation as Potential NPC Therapeutics

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Several lines of evidence suggest that β-cyclodextrin (β-CD) derivatives are able to initiate the efflux of accumulated unesterified cholesterol from the endosomal/lysosomal compartments of NPC1−/− cells and a murine model of NPC disease. Upon treatment of NPC1−/− 7 day old mice with various β-CD derivatives, unesterified cholesterol levels normalized in many tissues, with accompanying decreases in cholesterol synthesis and increases in average lifetime. Unfortunately, these effects are temporary and require repeated injections or continuous infusions to produce more durable responses. A growing family of β-CD polymers have been developed in the Thompson Lab in an effort to boost the efficacy and improve the pharmacokinetics of β-CD administration to make it a more palatable option as a potential NPC therapeutic. Our main effort has focused on the preparation of Pluronic-based polyrotaxanes that have been non-covalently threaded with either β-CD or HP-β-CD onto the central block of these PEG-PPG-PEG triblock copolymers. Eight different analogs, using Pluronic cores with different relative block lengths and with molecular weights ranging between 1.9 - 12.6 kD, have been synthesized. These compounds carry multiple copies of β-CD or HP-β-CD as shown by 1H NMR, gel permeation chromatography/multi-angle light scattering, and analytical ultracentrifugation analysis. A second class of β-CD polymers based on a PEG-PVA pendant polymer structural motif have also been developed and shown to carry multiple copies of β-CD derivatives. The release rate of the β-CD monomer precursors from these materials has also been shown to be controllable through structural modification. Preliminary data obtained in collaboration with the Storch Lab suggests that these compounds substantially reduce filipin staining in NPC2−/− fibroblasts.
Normalization of Cholesterol Homeostasis by 2-Hydroxypropyl-β-Cyclodextrin in Neurons and Glia from Niemann-Pick C1-deficient Mice

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Niemann-Pick C (NPC) disease is an inherited, progressive neurodegenerative disorder caused by mutations in the NPC1 or NPC2 gene that result in an accumulation of unesterified cholesterol in late endosomes/lysosomes (LE/L) and impaired export of cholesterol from the LE/L to the endoplasmic reticulum (ER). Recent studies have demonstrated that administration of cyclodextrin (CD) to Npc1-/- mice eliminates cholesterol sequestration in the LE/L of many tissues including the brain, delays the onset of neurodegeneration, and increases the life-span of the mice. We have now investigated cholesterol homeostasis in NPC1-deficient cells (glia cells and neurons) of the brain in response to different doses CD. Primary cultures of neurons and glial cells from Npc1-/- and Npc1+/- mice were incubated for 24 h with 0.1 to 10 mM CD after which survival and cholesterol homeostasis were monitored. Although 10 mM CD was profoundly neurotoxic, and altered astrocyte morphology, 0.1 and 1 mM CD were not toxic and both doses of CD effectively mobilized stored cholesterol from the LE/L as indicated by filipin staining. Importantly, 0.1 mM CD did not modulate parameters of cholesterol homeostasis in Npc1+/- neurons or astrocytes. However, 0.1 and 1 mM CD altered cholesterol homeostasis in opposite directions. Overall, the data suggest that 0.1 mM CD releases cholesterol trapped in LE/L of neurons and astrocytes and increases cholesterol availability at the ER, consistent with the effective dose of CD in the brain achieved by the Dietschy lab in intact mice. On the other hand, 1 mM CD primarily extracts cholesterol from the plasma membrane and reduces ER cholesterol. These studies in isolated Npc1-/- neurons and astrocytes establish a dose of CD (0.1 mM) that would likely be beneficial in NPC disease.
Use of Cyclodextrin in Two Brazilian Girls With Niemann-Pick Type C – Intrathecal Report
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We report the use of cyclodextrin in two Brazilian girls with Niemann-Pick type C (NPC). They started treatment with cyclodextrin by intravenous infusion twice a week in January 2010. After one year, they had significantly lower scores for NPC Clinical Severity Scale (NIH) and improves brain metabolism measured by PET-scan. In May 2011 there was a change of treatment. The cyclodextrin was administered intravenously once a week, and intrathecally, twice per month. We present the results after one year of the intrathecal treatment of two girls, describing the evolution of severity scores for NPC and changes in brain metabolism (PET-scan). We also compared the two treatments (intravenous and intrathecal), emphasizing safety and toxicity of intrathecal cyclodextrin.
Intrathecal cyclodextrin ameliorates neurological dysfunction and improves Purkinje cell survival in presymptomatic and postsymptomatic cats with Niemann-Pick Disease Type C

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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 have identified quantitative clinical and biochemical measures of disease severity and have used these markers to evaluate therapeutic efficacy.

We completed a miglustat therapy trial, and, by Fall 2012, will complete a hydroxypropylßcyclodextrin (HPßCD) therapy trial. Previously, we evaluated cats following intrathecal administration of 120 mg HPßCD. We identified that intrathecal administration of HPßCD 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 also identified a dose-related toxic effect of HPßCD on hearing function which had not been described in any other species.

Our next goal was to determine the minimum dose of intrathecal HPßCD sufficient for ameliorating neurological signs of NPC disease and the minimum dose resulting in altered hearing threshold. We administered intrathecal HPßCD at the following doses: 60 mg, 30 mg, 15 mg, 7.5 mg, and 3 mg. Therapy was begun at 3 weeks of age, prior to the onset of clinical signs. Cats showed significant to nearly complete amelioration of clinical signs at least up to 24 weeks of age (an age when untreated cats died). Lowering the dose of intrathecal cyclodextrin also resulted in amelioration of hearing dysfunction. Finally, we treated a cohort of cats at 16 weeks of age, an age at which significant neurological dysfunction was already present. These cats showed a slower onset of more severe neurological dysfunction and an increase in lifespan.
Niemann-Pick Type C disease (NPC) is a lethal, autosomal recessive disorder caused by mutations in the NPC1 and NPC2 cholesterol transport proteins. NPC’s hallmark symptoms include an accumulation of unesterified cholesterol and other lipids in the late endosomal and lysosomal cellular compartments, causing progressive neurodegeneration and death. Although the age of onset may vary in those affected, NPC most often manifests in juveniles, and is usually fatal.

In this study, we researched the effects of various compounds, many of which modify the epigenetic control of NPC1/NPC2 gene expression, in lowering the otherwise harmful elevated intracellular cholesterol levels in NPC cells. Our studies utilized a previously described image analysis technique, which allowed us to make quantitative comparisons of the efficacy of these drugs in lowering cholesterol levels. We investigated the effects of numerous compounds (SAHA/Vorinostat, LBH589/Panobinostat, β-cyclodextrin, rapamycin, decitabine, chloroquine, and chlorpromazine) on a compound heterozygote NPC1 mutant cell line and here report their efficacy. Of the drugs analyzed, several were found to significantly lower the relative amount of unesterified cellular cholesterol, indicating that, in the future, they may potentially be useful in treating NPC. Surprisingly, others were found to increase cellular cholesterol levels. We also studied combinations of successful compounds with β-cyclodextrin; significant cholesterol-lowering activity enhancements were observed in these treatments.
The Repurposing of vorinostat for the Treatment of Niemann Pick Type C
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Repurposing a previously approved drug provides the opportunity to reduce the time and costs to initiate clinical trials for a new disease indication by leveraging the known pharmacology, pharmacokinetics, and toxicology of the approved drug. In the case of Niemann Pick Type C (NP-C) repurposing a FDA approved drug would be a significant benefit for patients and to the developer. Using human NP-C fibroblast cells, two laboratories recently identified vorinostat, an FDA approved HDAC inhibitor (HDACi), to restore cholesterol homeostasis and reverse the dysregulation of the majority of the HDAC genes.

Vorinostat is a non-selective HDAC isoform inhibitor that in addition to its improvement of lipid metabolism may have several complementary pharmacological effects on neurological and inflammatory processes in the pathology of NP-C. HDACi have been shown to correct errant gene expression, ameliorate the progression of disease, and restore absent synthetic or metabolic activities for a diverse group of cancer and non-cancer disorders. In the case of mutated gene products with partial yet insufficient activity, as causes disease for many NP-C patients, HDACi treatment provides the opportunity to treat with increased transcriptional competence of a patient's own DNA. This pharmacological strategy treatment is well suited to rare diseases such as NP-C where the complexities of neurological symptoms make gene therapy, enzyme replacement or drugs that cannot penetrate the blood brain barrier difficult for development.

Vorinostat is an interesting drug for the treatment of NP-C because in laboratory studies, it not only corrects the basic NP-C metabolic defect of cholesterol homeostasis but also has complementary pharmacological activities that may benefit patient quality of life. Vorinostat may provide relief from progression of neurodegenerative symptomatology and inflammatory activity. Brain processes such as synaptic plasticity and memory may be improved by vorinostat treatment. The anti-inflammatory activity of vorinostat may improve the function of the brain and lungs, a serious complication of the disease. This may be attributed to the fact that vorinostat inhibits more than one HDAC isoform. HDACi have been shown to increase levels of regulatory T-cells (Tregs) in models of inflammation and auto-immunity. Studies from laboratories examining the effects of vorinostat inhibition on abnormal neurological and inflammatory activity report the association HDAC1, HDAC2, HDAC3, HDAC6 and HDAC7 as the cause. In the past several months the potential therapeutic application of vorinostat was shown for the rare diseases adrenoleukodystrophy, frontotemperol dementia, Gaucher’s and Huntington’s.

A data package compiling the supporting data of vorinostat pharmacology (cancer and non-cancer), pharmacokinetics, toxicology (approximately 750 references) and previous human experience (>1,000 patients) has been completed as part of the pending pre-IND meeting at the FDA. The established safety history of vorinostat is an important factor when designing clinical studies treating young NP-C patients. Vorinostat has been administered by oral and intravenous routes and has been shown to be brain penetrative from studies in humans and animals. Pediatric cancer patients have also benefited from vorinostat treatment and provide a basis for dosing paradigms in the treatment of NPC patients. The anticipated side-effect/adverse event occurrences are: fatigue, nausea, hematological. Vorinostat has pharmacological attributes that support its entry into clinical trials given the short life span of NP-C patients who cannot wait for the development and approval of a new drug.

Clinical protocols are awaiting FDA evaluation and comments. These studies will use the comprehensive metabolic panel, CBC, NP-C specific biomarkers from plasma and CSF samples, plasma chitotriosidase and cytokines to monitor the pharmacological effect of vorinostat treatment as well as the subjective NP-C severity scale. Vorinostat clinical trial materials will be provided by Merck Pharmaceutical without charge.
Identification of novel regulators of endosomal cholesterol trafficking

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Niemann Pick-C disease (NP-C) is characterized by cholesterol accumulation in late endosomes (LE) and lysosomes (LY). NP-C is caused by defects in either of two proteins, NPC1 and NPC2. NPC1 is a LE/LY membrane protein with a putative “sterol sensing domain” whereas NPC2 is a small, soluble protein that binds and transports cholesterol in the lumen of the LE/LY. Elegant recent work has provided important insights into the movement of cholesterol within the lumen of lysosomes, and how cholesterol may be delivered to the limiting membrane of LE/LY. What molecules/pathways mediate the transfer of cholesterol from LE/LY to the plasma membrane or the endoplasmic reticulum (ER)?

We have recently demonstrated that the oxysterol binding protein (OSBP)-related protein 5 (ORP5) plays a role in cholesterol trafficking from LE/LY to the ER. Through a targeted RNAi screen, we have now identified Hrs/VPS27 and VPS4/SKD1 as novel regulators of endosomal cholesterol trafficking. Interestingly, none of the other components of the ESCRT pathway is required for the exit of cholesterol from endosomal compartments. I will discuss how these factors may regulate cholesterol trafficking, and their possible interactions with NPC1, NPC2 and ORP5.

References:

Ryanodine receptor antagonists adapt NPC1 proteostasis to ameliorate lipid storage in Niemann-Pick type C disease fibroblasts

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Niemann-Pick type C disease is a lysosomal storage disorder most often caused by loss-of-function mutations in the \textit{NPC1} gene. The encoded multipass transmembrane protein is required for cholesterol efflux from late endosomes and lysosomes. Numerous missense mutations in the \textit{NPC1} gene cause disease, including the prevalent I1061T mutation that leads to protein misfolding and degradation. Here, we sought to modulate the cellular proteostasis machinery to achieve functional recovery in primary patient fibroblasts. We demonstrate that targeting endoplasmic reticulum (ER) calcium levels using ryanodine receptor (RyR) antagonists increased steady state levels of the NPC1 I1061T protein. These compounds also promoted trafficking of mutant NPC1 to late endosomes and lysosomes, and rescued the aberrant storage of cholesterol and sphingolipids that is characteristic of disease. Similar rescue was obtained using three distinct RyR antagonists in cells with missense alleles, but not with null alleles, or by over-expressing calnexin, a calcium-dependent ER chaperone. Our work highlights the utility of proteostasis regulators to remodel the protein-folding environment in the ER to recover function in the setting of disease-causing missense alleles.
Studying the effect of SAHA treatment on NPC1 protein life-time.

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Previously, we have shown that treating the human skin fibroblast of NPC1 patients with a family of histone deacetylase inhibitors (HDACi) at sub-micromolar concentration corrects the NPC1 phenotype. This effect was observed in two different NPC1 mutant fibroblasts, but not in NPC2 cells. However, although the two NPC1 mutant cells were from different patients, both had one common mutation at I1061T (GM03123 with compound heterozygous and GM18453 with homozygous mutation). We further studied the effect of one specific HDACi- SAHA on multiple additional NPC1 human fibroblasts cells from the patients carrying different type of mutations (courtesy Dr. Denny Porter, NIH). Our results indicated that SAHA is effective in reducing NPC1 phenotype in eight new mutant cell lines tested but to the varying degrees depending on severity with more severe phenotype being corrected to lesser extent and vice-versa. We had observed that decrease in cholesterol accumulation was accompanied by increase in NPC1 protein levels as seen by Western blot. Hence we are now measuring the NPC1 protein life-time in absence and presence of SAHA. New data will be presented and the results will help in understanding the therapeutic consequence of the drug treatment.