

7th National NCL-Congress

Informal Meeting of participants that have arrived one day earlier

Date: Wed, 1 October 2008
Time: 19:00
Dinner-location: Speicher 52 (restaurant of the Hamburg Marriott Hotel)

Date of congress: Thu, 2 October 2008
Time: approx. 10:00 – 17:00
Congress-location: Hamburg Marriott Hotel
 ABC-Straße 52
 20354 Hamburg, Germany
 Salon A

Preliminary agenda

- 10:00 – 10:15 Welcome of NCL-Foundation (Dr. Frank Stehr, NCL-Foundation)
- 10:15 – 10:45 A chemical biology approach to understanding autophagy pathway defects in JNCL (Dr. Susan Cotman, Massachusetts General Hospital, Boston, USA)
- 10:45 – 11:15 Phenotype and therapeutical trials in mouse models of CLN3 (Prof. Klaus Rütter, Charité, Berlin, Germany)
- 11:15 – 11:45 Human fetal neuronal stem cells as potential therapy for NCL (Prof. Robert Steiner, Child Development & Rehabilitation Center, Portland, USA)
- 11:45 – 12:15 Studies on JNCL (Prof. Beverly Davidson, University of Iowa, Iowa City, USA)
- 12:15 – 12:45 Novel roles for glia in NCL pathogenesis? (Prof. Jon Cooper, King's College London, London, GB)
- 12:45 – 13:45 Lunch break
- 13:45 – 14:15 Moving toward therapies for JNCL (Prof. David Pearce, University of Rochester, Rochester, USA)
- 14:15 – 14:45 AAV-mediated Gene Therapy for the CNS Manifestations of the Lysosomal Storage Disorders (Prof. Ronald Crystal, Weill Cornell Medical College, New York, USA)
- 14:45 – 15:00 Yeast as a model for Batten Disease: Roles for Btn1 and Btn2 in endosome-Golgi protein retrieval (Prof. Jeffrey E. Gerst, Weizmann Institute of Science, Rehovot, Israel)
- 15:00 – 15:15 Prestwick Chemical Library, a SOSA concept (Dr. Marie-Louise Jung, Prestwick Chemical, Illkirch, France)
- 15:15 – 15:45 Coffee-break
- 15:45 – 17:00 Podiums discussion & Summary
- 17:00 – 18:30 Meeting of the Scientific board
- 19:00 – 22:00 Dinner

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Abstracts

1. A chemical biology approach to understanding autophagy pathway defects in JNCL (Dr. Susan Cotman, Massachusetts General Hospital, Boston, USA)

Cln3^{Δex7/8} knock-in mice and CbCln3^{Δex7/8} cerebellar cell lines were generated that harbor the common mutation causing most cases of Juvenile Neuronal Ceroid Lipofuscinosis (JNCL). We are using these genetically accurate models to identify the earliest trigger of the JNCL disease cascade in order to establish novel therapeutic targets. Previous studies have demonstrated that deficient autophagy pathway trafficking is an early consequence of the common Cln3 mutation and that the hallmark JNCL feature, accumulation of subunit c of the mitochondrial ATP synthase, arises due to deficient autophagy pathway flux. Using CbCln3^{Δex7/8} cells, we have now developed an assay that measures the autophagy pathway defect caused by Cln3 mutation. With the aim of identifying the key molecular pathways involved in the JNCL disease process, we have employed the autophagy assay in high-content, cell-based chemical genetic screening studies. Compounds were identified that modulate the GFP-LC3 autophagosomal marker more or less dramatically in homozygous CbCln3^{Δex7/8} knock-in cells compared to wild-type cells, suggesting that the pathways the hit compounds target are altered by the common JNCL mutation. Follow-up studies are being undertaken to test the hypotheses generated by our screen. Our data support the notion that ion regulation is significantly altered by the common JNCL mutation, and we demonstrate that modulation of ion channels strongly influences disease phenotypes. Future studies will further delineate the role of ion regulation in the demise of NCL neurons, testing the hypothesis that targeting ion channels may provide therapeutic benefit in JNCL.

2. Phenotype and therapeutical trials in mouse models of CLN3 (Prof. Klaus Rütger, Charité, Berlin, Germany)

Purpose: To analyze the retinal phenotype of different mouse strains carrying the Cln3^{Δex7/8} mutation. In addition, first steps of therapeutic interventions using an antioxidant (α-tocopherol) and neural stem cells are described.

Methods: The retinal function of Cln3^{Δex7/8} knock-in mice bred on a CD1 and a C57Bl/6-background has been analyzed by electroretinogram recordings and histology. The therapeutic trials have been performed on mutant mice with a CD1 background. One group was treated by intraperitoneal injections of α-tocopherol (beginning with 2 injections followed by 1 injection per week for 11 weeks, control: olive oil). Another group was treated by intraretinal injections of neural stem cells at the age of 9 months. As a control, sham injection was performed at the fellow eye.

Results: At the age of 5 months mutant mice on a C57Bl/6 background showed a reduction of some ERG-components which was not the case in mice with a CD1 background. However, at later stages all mutants showed essentially the same phenotype irrespective of the genetic background. There was a reduction in both rod and cone function. The functional deficit happens mainly in the middle in inner retinal layers. Interestingly, whereas mice with the CD1-background became sick at 12 to 13 months of age, mice on the C57Bl/6 background were more viable. In contrast to non-treated animals, mice treated with α-tocopherol showed a cone response which was not discernable between wt and ki-mice. However, this effect was also observed in animals treated with the vehicle. In contrast, eyes injected with cells showed slightly reduced ERG-potentials when compared with mice treated with vehicle. In addition, the injection itself had a negative effect on the ERG-potentials.

Discussion: $Cln3^{\Delta ex7/8}$ knock-in mice display comparable phenotypes irrespective of the genetic background (i.e. CD1 or C57Bl/6). However, mice on a C57Bl/6 were more viable than mice on a CD1 background. There are indications that antioxidative agents such as α -tocopherol may have a positive effect on retinal function in ki-mice, but this effect might also be related to the vehicle (olive oil) or might even be a secondary effect of the ip-injection. First transplantation experiments with neural stem cells provided no evidence for functional improvements, but provide a reference for further cell-based therapeutic approaches using genetically engineered neural stem cells to target neuroprotective factors to the retina of $Cln3^{\Delta ex7/8}$ knock-in mice.

Supported by NCL-Foundation

3. Human fetal neuronal stem cells as potential therapy for NCL (Prof. Robert Steiner, Child Development & Rehabilitation Center, Portland, USA)

A Phase I clinical trial of purified human fetal neural stem cells in infantile and late infantile NCL was initiated by Stem Cells, Inc. at Oregon Health & Science University (OHSU) in 2006. The trial was approved for six children. Enrollment has been completed. The cells were delivered via a neurosurgical approach. All children recovered uneventfully from surgery and returned home. One death occurred in the trial, 11 months after the procedure. Brief preclinical data supporting the trial will be presented. Inclusion and exclusion criteria will be discussed, as will an overview of the trial procedures. Ethical issues will be mentioned, and possible future implications for NCL including JNCL will be presented.

4. Studies on JNCL (Prof. Beverly Davidson, University of Iowa, Iowa City, USA)

Juvenile neuronal ceroid lipofuscinosis is a severe inherited neurodegenerative disease resulting from mutations in $CLN3$ (ceroidlipofuscinosis, neuronal 3, juvenile). We recently generated a knock-in reporter mouse to elucidate $CLN3$ expression during embryogenesis and after birth and to correlate expression and behavior. In embryonic brain, expression appeared in the cortical plate. In postnatal brain, expression was prominent in the cortex, subiculum, parasubiculum, granule neurons of the dentate gyrus, and some brainstem nuclei. In adult brain, reporter gene expression waned in many areas but remained robust in vascular endothelia and the dentate gyrus. Cells from this model provide another opportunity to assess cellular physiology in the absence of $CLN3$, which we hope will help elucidate its function. We will discuss recent findings in cells cultured from this knockout model of $CLN3$ deficiency.

5. Novel roles for glia in NCL pathogenesis? (Prof. Jon Cooper, King's College London, London, GB)

Reactive changes accompany neuron loss in all forms of Neuronal Ceroid Lipofuscinosis (NCLs, Batten disease). However, these events do not occur globally, but display remarkable selectivity, especially early in pathogenesis. In human autopsy material the extent of astrogliosis, microglial activation and neuron loss differs markedly in different regions of the CNS, with more astrogliosis evident where neurons are better preserved and more activated microglia where neuron loss is more pronounced. Characterizing the relationship between astrocytes, microglia and neuron loss in mouse models of NCL has revealed a complex relationship between these three cell types and our recent data point towards novel roles for astrocytes at the synapse.

Although similar events may occur in each subtype, the sequence in which they happen during disease progression differs markedly between forms of NCL. In JNCL, an early glial activation precedes neuron loss by many months, but appears to be attenuated with incomplete morphological transformation of both astrocytes and microglia. These data raise the question that glia may be dysfunction in JNCL and we have begun exploring this possibility in vitro. Pure microglial or astrocyte cultures and neuron co-cultures derived from Cln3 deficient mice provide a powerful model system to investigate the role of neuron-glia interactions in JNCL pathogenesis. Our preliminary data provide the first evidence that glial biology may be compromised in JNCL.

6. Moving toward therapies for JNCL (Prof. David Pearce, University of Rochester, Rochester, USA)

Juvenile Batten disease is a progressive neurodegenerative disease. The brain is exquisitely complex in regard the processes that regulate neuronal function for multiple cell types in different neuro-anatomic regions. Using a Cln3 loss of function mouse model we have been characterizing the decline in vision and in motor and cognitive function. At the biochemical level we have used several models to establish changes at the cellular to be targeted for correction. This combined approach has revealed many potential pathological events that we aim to target for correction. Currently we are at a point with our research that has tested two distinct approaches at treatment in the Cln3 loss of function mouse. First, we have alleviated deteriorating motor function through suppression of the autoimmune response by genetic and drug mediated approaches. Second, by targeting an overactive receptor system in the brain with a drug, we have again alleviated deterioration in motor function.

7. AAV-mediated Gene Therapy for the CNS Manifestations of the Lysosomal Storage Disorders (Prof. Ronald Crystal, Weill Cornell Medical College, New York, USA)

Approximately 50% of the lysosomal storage disorders have a CNS component, presenting a major therapeutic challenge because the blood-brain barrier obviates the use of a systemic protein-based approach with recombinant lysosomal enzymes. Using late infantile neuronal ceroid lipofuscinosis (LINCL, a neurodegenerative, autosomal recessive lysosomal storage disorder caused by mutations in the CLN2 gene, resulting in tripeptidyl peptidase deficiency) as a model, we have developed a strategy to treat the CNS manifestations of these disorders using direct CNS administration of adeno-associated virus (AAV) vectors coding for the deficient lysosomal enzyme. We will provide an update on: (1) the challenge of developing quantitative clinical phenotypes to assess CNS therapy and the development of strategies to quantify the CNS manifestations of these disorders in mice; (2) the clinical study of n=10 children with LINCL using AAV serotype 2 coding for CLN2; (3) murine and non-human primate efficacy and toxicology assessment of CNS administration of AAVrh.10, a non-human primate-derived AAV serotype; (4) the use of AAVrh.10 to deliver proteins on a persistent basis to the retina epithelium; and (5) strategies to adopt this paradigm to treat JNCL.

8. Yeast as a model for Batten Disease: Roles for Btn1 and Btn2 in endosome-Golgi protein retrieval (Jeffrey E. Gerst, Vydehi Kanneganti, and Rachel Kama, Weizmann Institute of Science, Rehovot, Israel)

We employ yeast as a model system to understand the role of conserved proteins involved in Batten disease and related lysosomal storage disorders in humans. Neuronal ceroid lipofuscinoses (NCLs), including the juvenile onset form known as Batten disease, are characterized by abnormal accumulation of autofluorescent material in lysosomes.

Thus, the study of NCLs requires an in-depth understanding of the intracellular trafficking pathways that deliver proteins and lipids from Golgi to lysosomes and back via the endosomal compartments. Defects in protein delivery to the lysosome via endosomes may result in organelle expansion, cellular degeneration, and onset of the disease state.

We have demonstrated that Btn2, a yeast ortholog of Hook1 and a potential Batten disease-related protein, mediates protein retrieval from late endosomes (LEs) to the Golgi in yeast (Kama et al, 2007, Mol. Cell. Biol. 27:605-621). Btn2 resides on a late endosomal compartment and interacts with known proteins involved in LE-Golgi protein retrieval. These include SNAREs of the endosomal SNARE complex, a sorting nexin, and components of the retromer complex that mediates LE-Golgi protein sorting.

Importantly, the deletion of BTN2 in yeast leads to specific defects in the trafficking of a number of cargo proteins that pass through the LE. These include Yif1, a Golgi protein whose retrieval from the LE to the Golgi is perturbed in the absence of BTN2, as seen earlier (Chattopadhyay et al, 2003, BBRC 302:534-538). In addition, Kex2, a subtilisinlike protease of the late secretory pathway that traverses the LE to return to the Golgi, is also mislocalized to the LE in the absence of BTN2. Importantly all defects in protein trafficking in btn2 Δ cells are phenocopied by mutations in retromer and other genes known to be involved in LE-Golgi protein retrieval.

We now show that a deletion in BTN1, which encodes the ortholog of the known Batten Disease associated gene, CLN3, leads to identical defects in LE-Golgi sorting.

Moreover, Btn1 localizes to the Golgi and, thus, acts in a distal fashion to regulate protein retrieval. The mechanism underlying how Btn1 regulates LE-Golgi retrieval will be discussed. Together, our results suggest that Batten disease (and, perhaps, other NCLs) result from defects in endosomal protein sorting.

9. Prestwick Chemical Library, a SOSA concept (Dr. Marie-Louise Jung, Prestwick Chemical, Illkirch, France)

Prestwick Chemical, founded by Prof. C.G. Wermuth, is a premium provider of medicinal chemistry services. We also offer innovative smart screening libraries and research tools to pharmaceutical & biotechnology companies, as well as academic research laboratories and institutions.

Contract research services in medicinal chemistry: Hit validation – Lead optimisation – Ligand profiling

Prestwick's team of well trained medicinal chemists develops innovative medicinal chemistry strategies tailored to clients' needs. They are supported by state-of-art computational ligand design, including large scale virtual screening and take into account ADME/Tox as well as selectivity issues. This helps to minimize time and maximize results (in average 18 months from inception to acceptable drug candidate). Possible contracts include "no string attached" programs (FTE based), objective driven programs (minimum base fee + milestones) and risk sharing collaborations (with or without financing).

Different screening libraries (compound collections):

Our Smart Libraries have been designed to ensure maximal chemical diversity, possibly to access new IP while remaining within reach of both low and high throughput screening processes. They are constantly updated and improved. Our clients have reported high quality hit generation rate.

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