The safety and efficacy of 2-hydroxypropyl-beta-cyclodextrin (cyclodextrin) was evaluated in the cat model of Niemann Pick type C (NPC) disease [1]. Direct administration of cyclodextrin into the spinal fluid at the base of the head of NPC cats prevented the onset of tremors and incoordination and corrected cholesterol and sphingolipid storage. However, hearing loss was identified as a negative effect[1]. These studies were published in February 2015 and have provided critical data on efficacy and safety of drug administration directly into the central nervous system that have proven important in advancing cyclodextrin into clinical trials [2].

NNPDF funding also allowed us expand our breeding colony (six more female heterozygotes) to allow for the production of additional affected cats (n=20 over two years). This was done and we now can produce over 20 affected cats per year. The following results were obtained using these additional cats in addition to the work described above:

1. <u>To compare the effect of intrathecal administration of cyclodextrin at the lumbar cistern (lower back) to data collected from administration at the cerebellomedullary cistern (base of head).</u>
   Cats that received cyclodextrin in the lower back did not do as well as those treated at the base of the head. When cyclodextrin concentrations were determined, it was found that brain concentrations were approximately 4 times lower in cats that received cyclodextrin in the lower back compared to those that received it at the base of the head. This suggests that higher doses of cyclodextrin will be necessary in children receiving lumbar injections.
2. <u>To examine whether systemic delivery of a histone deacetylase inhibitor (HDACi) slows progression of neurodegeneration, prolongs survival, and alters biomarker profile.</u> Studies performed on cat cells showed that HDACi administration did not result in improvement in cholesterol storage. This is hypothesized to be due to the mutation present in the cat model. No benefit is expected from the use of HDACi in this model, and therefore the studies were not performed in cats.
3. <u>To develop and validate biomarkers of disease severity.</u> Our published work with Dr. Ory identified robust cholesterol-derived NPC disease lipid biomarkers: cholestane-3β,5α,6β-triol ("triol"), a cholesterol oxidation product that is elevated 10-fold in the plasma of NPC subjects, and 24(S)-hydroxycholesterol (24(S)-HC), an enzymatically-generated oxygenated cholesterol that is reduced in the plasma of NPC subjects[3]. Cats receiving cyclodextrin exhibited a significant dose-dependent increase in 24(S)-HC in the spinal fluid 3 days following the initial dose ($p<0.01$). Consistent with the 24(S)-HC response, CSF cholesterol ester species were elevated 1.3-1.6-fold ($p\leq0.05$) in the treatment groups. Plasma triol concentrations, which primarily reflect production of this oxysterol in peripheral tissues [3], increased linearly with age (i.e., disease-severity) in the control animals.

   Calbindin is an intracellular calcium binding protein that is commonly used to label Purkinje cells in NPC disease. When Purkinje cells die, calbindin staining decreases and we hypothesized that the calbindin released from dying Purkinje cells during progressive NPC disease would result in elevations in CSF calbindin concentrations. Normal, untreated NPC, and NPC cats treated with cyclodextrin were evaluated over time. These studies showed that calbindin CSF concentrations increased with disease progression in NPC cats and that presymptomatic therapy with HPßCD resulted in concentrations indistinguishable from normal levels. Thus, calbindin levels in the CSF can be used as a measure of ongoing Purkinje cell loss and disease progression and will be utilized as a biomarker in post-symptomatically treated cats in the experiments outlined below.

<u>Publications resulting from funded studies:</u>

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5. Vite, C.H.*, et al.* Intracisternal cyclodextrin prevents cerebellar dysfunction and Purkinje cell death in feline Niemann-Pick type C1 disease. Sci Transl Med 7, 276ra226, 2015.

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4. Tortelli, B., *et al.* Cholesterol homeostatic responses provide biomarkers for monitoring treatment for the neurodegenerative disease Niemann-Pick C1 (NPC1). *Hum Mol Genet* **23**, 6022-6033 (2014).