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Progress Report

Lay Summary

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). We identified 12 pathways that exacerbate lethality in yeast that can potentially be reversed in human cells as a novel therapeutic strategy. One novel target is a pathway that regulates gene expression; this is the homeostasis between histone acetylation (HAT) and histone deacetylation (HDAC). We demonstrated that this HAT-HDAC pendulum is dysregulated in NPC disease with an excess of HDAC activity. Therefore, 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. SAHA exhibits great potential as a therapy for NPC patients since it is already approved by the FDA to treat cutaneous T-cell lymphoma and is readily available as oral tablets. As an NNPDF Peter Pentchev Fellow, I am determining the therapeutic efficacy of SAHA by translating our *in vitro* results in fibroblasts to *in vivo* results in animal models.

We have optimized dosage and delivery of SAHA in the *Npc1nmf164* mouse model of NPC disease and critically determined that SAHA crosses the blood-brain barrier in P21 mice, an age that is comparable to a juvenile NPC patient where the blood-brain barrier is not readily permeable. This treatment regime resulted in micromolar concentrations in the plasma and brain of mice; these are the same concentrations that were previously used to rescue lipid accumulation in NPC patient fibroblasts. This result predicts that SAHA treatment will rescue lipid accumulation in the brain and liver of the mice. We indeed determined that SAHA ameliorated cholesterol and sphingolipid accumulation in the liver at an adolescent stage of the *Npc1nmf164* mouse model of NPC disease. The rescue of cholesterol accumulation occurred via an improvement in cholesterol esterification, a process that is defective in NPC disease. Interestingly, we determined that the mechanism by which SAHA has a therapeutic impact in the liver is not through increased expression of NPC1, thus we are currently pursuing other mechanisms to explain this candidate therapy.

In summary, we now have the evidence that SAHA is a viable therapeutic option in in the liver of an animal model and are now in the process of testing if it is also a therapeutic option in the brain by treating these mice at a later stage when they accumulate lipids in the brain. Also in the next year, comparable experiments will be conducted in the feline model of NPC disease using a dosage that is calibrated for the weight of a cat. These studies testing the therapeutic efficacy of SAHA in a mouse model and a feline model will fully determine the potential of SAHA as a therapy to treat children affected with NPC disease.