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Phenotypic Screening of Small Molecules for the Treatment of CLN3 Batten Disease

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Batten disease is a form of genetic neuronal ceroid lipofuscinosis (NCL) disorder that usually manifests in infancy to teenage years. Sub-types of Batten disease exhibit similar symptoms including seizures, loss of vision, epilepsy, cognitive and motor decline that progress to dementia, and premature death. In the U.S., Batten disease affects about three out of every 100,000 births. Batten disease is an inherited metabolic disorder, and all types are fatal except adult Batten disease. All NCLs are associated with lysosomal dysfunction and accumulation of autofluorescent ceroid lipofuscin and/or subunit c of mitochondrial ATP synthase. Subtype of NCLs occur when one of 13 different genes are mutated, wherein only two subtypes (CLN2 and CLN5) have approved clinical treatment options using gene therapy or enzyme replacement therapy. As CLN3 does not occur due to a loss-of-function mutation in an enzyme, genetic therapies are not an option. CLN3 disease cells are known to experience dysfunctional autophagy that was also seen in CLN3 disease patient-specific induced pluripotent stem cell (iPSC)-derived neurons and neural progenitor cells (NPCs) in addition to mitochondrial dysfunction. The CLN3 gene is also known to have anti-apoptotic properties via Bcl-2 upregulation, and controls calcium homeostasis, thereby regulating autophagy, which is a hallmark for CLN3 disease. Together, activation of autophagy and inhibition of apoptosis mediated by neuroprotective small molecules have potential therapeutic benefit for CLN3 disease. Flupirtine and retigabine are small molecules noted for their neuroprotective properties at higher concentrations. To minimize their side effects, a library of compounds was generated based on flupirtine and retigabine templates. Lead molecule was evaluated for *in vivo* pharmacokinetic properties. The effect of compounds to activate KCNQ2/3 (Kv7.2/Kv7.3) potassium channels was also evaluated. The synthesized compounds exhibited good potency in the phenotypic screen of iPSC-derived NPCs. Compound-2 showed good 'drug-like' pharmacokinetic profile. The compounds showed significantly reduced effect to activate Kv7.2/Kv7.3. We have synthesized analogues of flupirtine and retigabine and developed a robust protocol for phenotypic screening of these compounds in CLN3 patient iPSC-derived NPCs. Compounds possess enhanced potency and retain 'drug-like' pharmacokinetic properties. The lead compound has a reduced effect to activate KCNQ2/3 channels. Future directions include further evaluation of structure-activity relationship of the lead molecules and testing their potency for autophagy induction and inhibition of apoptosis. Future studies also include the evaluation of lead molecules in an in vivo model of CLN3 disease using *Cln3Δex7/8* mice.