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Progress in the Development of Small Molecule Therapeutics for the Treatment of Neuronal Ceroid Lipofuscinoses (NCLs)

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Abstract

The neuronal ceroid lipofuscinoses (NCLs) are a group of inherited and incurable neurodegenerative disorders primarily afflicting the pediatric population. Current treatment regimens offer only symptomatic relief and do not target the underlying cause of the disease. Although the underlying pathophysiology that drives disease progression is unknown, several small molecules have been identified with diverse mechanisms of action that provide promise for the treatment of this devastating disease. This review aims to summarize the current cellular and animal models available for the identification of potential therapeutics and presents the current state of knowledge on small molecule compounds that demonstrate in vitro and/or in vivo efficacy across the NCLs with an emphasis on targets of action.

Graphical abstract

INTRODUCTION

The neuronal ceroid lipofuscinoses (NCLs), commonly grouped together as Batten disease, are the most common neurodegenerative diseases of the pediatric population.^{1,2} The incidence of NCL is estimated at 1 in 12 500;³ however, this representation may not be accurate due to the rarity of the disease. Although rare, the disease often strikes multiple offspring in the same family that carry the defective NCL gene. Enzyme replacement therapy, stem cell therapy, gene therapy, dietary supplements, and small molecules have been tested in various cell and animal models and in humans. No cure for NCL has yet been realized; however, there is pharmacological intervention in clinical trials^{4–6} that can slow

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disease progression and/or improve quality of life.^{7–10} The need for the identification of new drug candidates that are disease-modifying in the NCLs is unmet and urgent, yet is hampered by the largely unknown pathophysiology of NCL progression.

The disease presents with early vision problems and/or seizures progressing to mental impairment, seizures of increased severity, progressive loss of sight and motor skills, and increasing spasticity. Children reaching the end-stages of the disease become blind, bedridden, spastic, and demented with poorly controlled seizures before expiring in their teens or early twenties.^{2,11,12} The NCLs progress through an accumulation of lipofuscin in body tissues. Fat, but primarily protein deposits (termed storage material), accumulates in the brain, retina, and other tissues, which specifically locate to the lysosomes.^{13,14} Accelerated apoptosis,^{15,16} impaired autophagy, and secondary destructive inflammation have been documented. Accumulation of subunit c of mitochondrial ATP synthase in lysosome-derived organelles is observed in NCLs; however, the exact biochemical mechanism of accumulation is unknown.^{14,17}

Most forms of NCL are autosomal recessive disorders, with the exception of some families with adult Kufs disease manifesting autosomal dominant inheritance.¹¹ The NCLs are linked to 13 genes; mutations in any of the genes named as CLN1–14 can lead to NCLs. Proteins encoded by the majority of the NCL genes (CLN1, CLN2, CLN3, CLN5, CLN7, CLN10, CLN12, and CLN13) are primarily localized to the lysosome. Some of these proteins are also known to have extralysosomal functions.^{18,19} Mutations in the CLN3 gene cause juvenile NCL (JNCL) and encode for the CLN3 protein, which has been localized to Golgi, lipid rafts and plasma membrane, and lysosomes. However, the overall function of the CLN3 protein is unknown; mutations lead to altered lysosomal pH, defective arginine transport, and malfunction in transport across lysosomal and vacuolar membranes.^{20–22} In CLN3 knockdown SH-SY5Y cells, calcium induced cell death was mediated by calsenilin, a calcium sensor known to be proapoptotic in neurons. Calsenilin binds to the C-terminal of CLN3, which is known to be cytoprotective, leading to a reduction in calsenilin induced cell death.²³ It has been proposed for JNCL that different receptors undergo dysregulation in an age dependent fashion as observed in *Cln3*^{ex1–6} mice. This same process has been observed in a mouse model of Huntington's disease, the cellular and molecular mechanism changing during disease progression.^{24,25}

Another common NCL variant is CLN2 disease or late infantile NCL (LINCL), attributed to a defective tripeptidyl peptidase I (TPP1) enzyme, the protein product of the healthy CLN2 gene. Infantile NCL (INCL) is the most severe form of NCL. Patients have a defective palmitoyl protein thioesterase 1 (PPT1) enzyme, caused by a mutated CLN1 gene.²⁶ PPT1 is responsible for the cleavage of palmitate from S-acylated proteins,²⁷ whereas TPP1 removes tripeptides from the N-terminal of small polypeptides.²⁸ However, the native substrates of these enzymes remain unidentified.¹ CLN4 disease is caused by mutations in the *DNAJC5* gene which encodes for the protein cysteine-string protein α (CSP α).²⁹ CSP α has been shown to play a role as a chaperone in folding of proteins and in synaptic vesicle exocytosis and endocytosis.^{30,31} The cathepsin D (*CTSD*, *CLN10*) gene in the physiological state codes for the protein cathepsin D, an aspartyl endopeptidase, which plays several roles in apoptosis. CLN10 disease is caused by mutation in the cathepsin D gene.^{1,18} Mutation in the

cathepsin F (*CTSF*, *CLN13*) gene leads to adult-onset NCL, also known as type B Kufs disease.³² Cathepsin F is a cysteine protease highly expressed in neurons and is shown to be involved in autophagy and proteasomal degradation.^{1,18} The functions of proteins encoded by genes *CLN5–9*, *CLN11*, *CLN12*, and *CLN14* are unknown.^{1,18} Some of the *CLN* proteins demonstrate interactions with each other, but their physiological relevance is unknown.¹

Apoptosis, among other cellular death mechanisms, has been proposed as one of the mechanisms of neuron death in the NCLs based on in vitro experiments.³³ One mechanism of survival for neurons in LINCL and JNCL is upregulation of the apoptotic regulator protein Bcl-2. Likewise the *CLN3* gene, mutated in the disease state, is known to exhibit antiapoptotic properties, exacerbating this effect by upregulation of the apoptosis suppressor Bcl-2.^{33–35} The gene that mutates in LINCL, *CLN2*, was also shown to be antiapoptotic; hNT neurons transduced with sense-*CLN2*-AAV2 virus showed protection against etoposide induced apoptosis, whereas NT2 cells transduced with antisense-*CLN2*-AAV2 showed an increased rate of apoptosis.³⁴ Mutations in *CLN1*,³⁶ *CLN5*, *CLN6*, and *CLN8* genes also result in accelerated apoptosis contributing to the manifestation of NCL at different ages.³⁷ Wild-type *CLN3* has also been implicated in down-regulating ceramide generation, a proapoptotic lipid second messenger.¹⁶ It was believed that the deposition of storage material in specific parts of the brain led to death of neurons. However, experiments conducted in a *CLN6* sheep model show no correlation between the deposition of storage material and death of neurons, suggesting that other mechanisms of cell death may also play a role.^{38,39} This also suggests that reduction in storage material may not be a therapeutic approach to treat Batten disease nor a reliable biomarker to measure treatment effect.

While the exact pathophysiology of the NCLs is unknown, the basic understanding of disease processes elucidated to date has allowed the creation of cellular and animal models that mimic the phenotype of deficient *CLN* genes. This in turn has led to the evaluation of several small molecule candidate therapeutics for use in the treatment of this devastating disease (Table 1).

CELLULAR MODELS

Several phenotypic models mimicking the NCL disease state in humans have been developed to aid drug discovery efforts and to help determine the underlying pathophysiology. Several researchers have reported the use of NCL patient lymphoblasts and fibroblasts, along with neurons derived from animal models of NCL disease.^{40–45} A phenotypic model of the human neuroblastoma cell line SH-SY5Y was developed using siRNA knockdown of the *CLN3* gene, the gene known to mutate in JNCL.⁴⁶ A similar model was developed in primary rat cortical neurons by *CLN3* knockdown using a siRNA based method.⁴⁷ Two cell models of LINCL and JNCL were created, where hNT neurons were transduced with AS-*CLN2*-AAV2 and Ad-AS-*CLN3*, resulting in loss of *CLN2* and *CLN3* expression, respectively.³⁴

Human induced pluripotent stem cell (iPSC) models have gained increasing attention, having the advantage that the majority of the genotypic and phenotypic changes observed in

human NCL patients are reciprocated by this model. Fibroblasts obtained from LINCL and JNCL patients were reprogrammed to iPSCs; however, no changes were observed in the Golgi or mitochondria in both CLN2 and CLN3 patient iPSCs, but lysosomal changes were observed without deposition of storage material and mitochondrial ATP synthase subunit c. The iPSCs derived from LINCL patients did, however, exhibit reduced TPP1 activity.⁴⁸

Neural progenitor cells (NPCs) generated in turn from iPSCs did exhibit differences in structure of the Golgi body and lysosomal membranes along with deposition of storage-like material different from the storage material observed in NCL patients; morphological anomalies in mitochondria were also observed. The NPCs obtained from CLN2 patients showed diminished TPP1 activity, while CLN3 patient NPCs showed slightly elevated TPP1 activity. Deposition of subunit c of mitochondrial ATP synthase was observed similar to NCL patients in both sets of NPCs.⁴⁸

Mature CLN2 and CLN3 patient neurons were also generated from NPCs. Mature neurons showed aberrations in the Golgi body, mitochondria, endoplasmic reticulum (ER), and lysosomal membrane. Storage material similar to NCL patients was also observed. An increase in disease progression was observed in the iPSC model when moving from iPSC to mature neurons.⁴⁸ However, iPSCs suffer from high degrees of variation between attempts to reprogram a single fibroblast line or between cells derived from different patients, complicating their use in early drug discovery screens. Finally, a yeast model for the study of NCL has been developed, as the organism contains a gene, *BTN1*, which is homologous to the human *CLN3* gene.⁴⁹

ANIMAL MODELS

Currently there are several animal models available for different types of NCLs including *Drosophila*,⁵⁰ mouse,^{51–54} sheep, cow, ferret, cat, horse, goat, pig, parrot, monkey, duck, and canine.^{51,54} Of particular significance are the PPT1 knockout mice models mimicking the CLN1 disease state, and the *Cln3*^{ex1–6} mimicking the CLN3 disease state, as the majority of small molecule therapeutic investigation for NCLs are performed using these models. Other mice models for CLN3 disease such as *Cln3*^{ex7/8} knockout, *Cln3*^{ex7/8} knock-in, and *Cln3*^{LacZ} β -galactosidase have also been developed.⁵⁵ Several other mice models are also available: three for CLN1, two for CLN2, and one each for CLN5–8 and CLN10.⁵²

PPT1 Knockout Mice as a Model for CLN1 Disease

PPT1 gene disruption was verified using Southern blot and immunoblotting and was confirmed by background levels of PPT1 enzymatic activity. A 50% mortality rate was observed in PPT1 knockout mice by 7 months of life, and seizures were observed from 3–4 months of age. Significant increase in autofluorescence was observed at 4 weeks of age, and granular osmiophilic deposits (GRODs) were readily observed along with thinning of the hippocampus, ballooning defragmentation of neurons, and apoptotic nuclear fragments.⁵⁶

Cln3^{ex1–6} Mouse Model

A *Cln3* gene disrupted mouse model in which several neuropathological changes are observed is now extensively utilized in the field of NCL disease.^{24,57} Disruption of the

CLN3 gene was confirmed using reverse transcription polymerase chain reaction (RT-PCR) and Southern blotting. Significant increase in accumulation of storage material in the neurons of *CLN^{-/-}* mice was observed. Autofluorescent lipopigments were detected at 3 months of age, and a progressive increase in quantity was observed. Autofluorescent lipopigment was distributed in several parts of the brain including the cortex, hippocampus, basal ganglia, and brainstem. A reduction in the brain mass was noted that was not statistically significant; however, a decrease in the cortex volume was also observed that was statistically significant. CLN2 protease activity was elevated in the brain similar to that activity observed in NCL patients.⁵⁷ α -Amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid (AMPA) and *N*-methyl-D-aspartate (NMDA)-type glutamate receptor dysfunction has been observed in this model as well as in JNCL patients.^{24,58}

SMALL MOLECULE THERAPEUTICS EVALUATED IN CLN3 DISEASE (JNCL)

Flupirtine (1), a non-opioid analgesic approved for the treatment of seizures in Europe, is known to be neuroprotective via the upregulation of Bcl-2 and antagonism at the NMDA receptor.^{34,59} Flupirtine reduced the rate of apoptosis in CLN1-, CLN2-, CLN3-, and CLN6-deficient NCL patient lymphoblasts. The compound also showed a neuroprotective effect in hNT neurons transduced with AS-CLN2-AAV2 and Ad-AS-CLN3 and from etoposide induced apoptosis in hNT neurons and hNT neurons transduced with sense-CLN2-AAV2. In addition, flupirtine rescued hNT neurons from NMDA activated neuronal apoptosis.³⁴ In a follow-up study to assess the effect of flupirtine in JNCL patients, a survey was conducted among the parents of patients who were undergoing treatment with flupirtine. The unified Batten disease rating scale (UBDRS) was used to assess five aspects of JNCL: physical ability, seizure activity, behavioral problems, functional capacity, and global clinical impression of disease. Parents reported a beneficial effect from flupirtine; however, statistical analysis showed no difference between the treatment and control groups. This study was not a clinical trial and was solely based on the survey filled out by the parents of JNCL patients.⁶⁰ The initial study reported that flupirtine was neuroprotective in vitro at >20 μ M, a concentration difficult to achieve and maintain within the human body.^{34,60}

1 Flupirtine **2 Amlodipine**

It is well established that elevated intracellular calcium levels induce apoptosis.¹⁶ Elevated levels of CLN3 protein, as opposed to deficient levels as seen in JNCL patients, have been shown to reverse apoptosis induced by abnormal intracellular calcium accumulation.⁶¹ In JNCL, CLN3 gene mutation causes calcium induced cell death.²³ In a primary rat cortical neuron model with CLN3 knockdown using siRNA, amlodipine (**2**) reversed the elevated calcium levels and thus prevented apoptosis.^{47,62} Amlodipine functions as a calcium channel blocker; however, follow-up studies on other calcium channel blockers have not been reported.

Hyperactivity of the AMPA receptor in cerebellar neurons in Cln3^{ex1-6} mice has been suggested as one of the biochemical dysfunctions of the NCLs.⁵⁸ The selective, noncompetitive AMPA receptor antagonist **3** (EGIS-8332) at 1 mg/kg, ip, improved motor coordination in 1 month-old Cln3^{ex1-6} mice.

3 EGIS- 8332

Higher doses of 3 mg/kg and 10 mg/kg, however, did not show any enhancement in motor coordination.⁶³ Compound **3** (1 mg/kg, ip) did not show any improvement in motor skills immediately after treatment but did show a delayed response after 4 days in 6- to 7-month-old Cln3^{ex1-6} mice. The compound did not improve the total neuronal count and was ineffective on astrocytic and microglial activation in cortex and the cerebellum.⁶⁴

4 Memantine

Anomalous glutaminergic activity has been observed in various mouse models and human patients of neurodegenerative disorders including infantile, late infantile, and juvenile NCL.⁶⁵⁻⁶⁹ Acute treatment with the NMDA receptor antagonist memantine (**4**) (1 mg/kg and 5 mg/kg, ip) showed no improvement in motor coordination in 1-month-old Cln3^{ex1-6} mice; however, 1 mg/kg memantine showed significant improvement in motor coordination after 1 day and 5 mg/kg showed a delayed response with an improvement in motor coordination after 4 days of treatment in 6- to 7-month-old Cln3^{ex1-6} mice. To assess the age-dependent effect, repeated doses of memantine (1 mg/kg) and **3** (1 mg/kg) were administered once a week for 4 weeks. Seven-month-old Cln3^{ex1-6} mice treated with compound **3**, in comparison to memantine treated mice, showed an enhanced performance on the rotarod test. Neither of the compounds showed an increase in neuron numbers in the thalamus or cerebellum. The authors suggested that acute inhibition of the NMDA receptor (a known mechanism of action of flupirtine (**1**) reviewed above) could serve as a therapeutic target for NCL.²⁴

Neurons isolated from *Ppt1*^{-/-} mice have shown NMDA receptor hyperfunction.⁶⁷ The effect of memantine was examined in 3-month-, 5-month-, and 7-month-old *Ppt1*^{-/-} mice using an accelerating rotarod. A dose of 10 mg/kg did not show any significant effect. A dose of 20 mg/kg did not show a significant effect in 3- and 5-month-old *Ppt1*^{-/-} mice; however, it did show a significant effect in motor learning in 7-month-old *Ppt1*^{-/-} mice. The authors conclude that further tests are required but memantine may be useful in INCL patients who have already developed disease symptoms.⁷⁰

Mice with the Cln3^{-/-}/μMT mutation were developed that were incapable of producing immunoglobulin G. The mice demonstrated ameliorated levels of neuroinflammation. At postnatal day 60 (P60), Cln3^{-/-}/μMT mice showed significantly improved motor

coordination as compared to $Cln3^{-/-}$ mice; however, this difference was not observed at P100 and P180. This $Cln3^{-/-}/\mu MT$ mutation was used as a model to assess the effect of immunosuppressant activity on reduction of neuroinflammation prior to the evaluation of small molecules.

5 Mycophenolate mofetil

Mycophenolate mofetil (**5**), an immunosuppressant acting by inhibiting inosine monophosphate dehydrogenase and thus B and T cell proliferation, was evaluated as a potential therapeutic in JNCL. Treatment with compound **5** resulted in a significant increase in motor coordination in $Cln3^{-/-}$ mice and reduced neuroinflammation at 60 mg/kg dose compared to untreated $Cln3^{-/-}$ mice.⁷¹ A phase II clinical trial has recently been initiated to determine the safety and tolerability of short-term mycophenolate mofetil administration in patients with JNCL.⁷²

Lithium, which has been shown to be neuroprotective,⁷³ albeit with an unknown mechanism of action, reduces autophagosomes in CLN3 mutant knock-in cerebellar cells (Cb $Cln3^{ex7/8/ex7/8}$). Lithium also reduced the accumulation of subunit c of mitochondrial ATP synthase and autofluorescence and prevented cell death induced by amino acid deprivation. The mechanism of action leading to the neuroprotective effect of lithium in Cb $Cln3^{ex7/8/ex7/8}$ cells was shown to be via IMPase inhibition.⁷⁴

6 *N*-acetylcysteine

CLN3 has been shown to have a protective effect against oxidative stress induced neuronal cell death in a *Drosophila* model.⁷⁵ Mutated CLN3 is responsible for reduced lysosomal arginine levels which would stimulate the urea cycle, increasing nitric oxide (NO) byproducts resulting in generation of reactive oxygen species and DNA damage. Increased levels of biomarkers including carbamoyl phosphate synthetase 1 (CPS1), 8-oxoguanine DNA glycosylase 1 (OGG1), and DNA polymerase β were observed in NCL patient lymphoblasts. Treatment with *N*-acetylcysteine (**6**), a known antioxidant, precursor, and analogue of glutathione, reduced expression levels of CPS1, OGG1, and DNA polymerase β in cells derived from Batten disease patients.⁴⁵ A pilot study was conducted in nine INCL patients to determine the beneficial effects of combination of cysteamine bitartrate and *N*-acetylcysteine. Decrease in the number and size of GRODs was observed after the first

posttreatment follow-up and in the subsequent follow-up GROD levels were undetectable indicating that the treatment had beneficial effects in patients.⁵

SMALL MOLECULE THERAPEUTICS EVALUATED IN CLN1 DISEASE (INCL)

N-(*tert*-Butyl)hydroxylamine (NtBuHA) (**7**) was found to be nontoxic to INCL patient lymphoblasts and fibroblasts, mimics PPT1 by cleaving thioester linkage in palmitoyl-CoA and palmitoylated proteins, reduces GRODs which correlate to ceroid depositions in the cells of INCL patients, ameliorates ER and oxidative stress, and suppresses oxidative stress mediated apoptosis.⁴⁴

7 *N*-tert-butyl hydroxylamine

Similarly, NtBuHA in *Ppt1*^{-/-} mice does not stimulate the production of methemoglobin. Unlike the structurally related compound hydroxylamine, the compound crosses the blood–brain barrier (BBB), depletes GRODs and ceroid deposition, and therefore reduces autofluorescence and apoptosis, preserving motor function and prolonging lifespan. While this compound represents one of the most beneficial small molecules ever evaluated in NCL patient cells and murine models, NtBuHA is only effective in depalmitoylating S-acetylated proteins and is unlikely to help in the depalmitoylation of N-palmitoylated proteins, as the palmitate is linked to the cysteine residues by an amide bond.⁴⁴

8 Cysteamine

Cysteamine (**8**), the simplest stable aminothiols, was shown to cleave palmitoyl-CoA in vitro.⁷⁶ A structure–activity relationship (SAR) of various aminothiols against PPT substrate accumulation was derived. The aminothiols were active at slightly alkaline pH and inactive at acidic pH. The sulfhydryl group was shown to be essential, as it functions to cleave the thioester linkage of the substrate. Cysteamine reduces the quantity of storage material present in INCL lymphoblasts; however, the closely related analogue phosphocysteamine (**9**) does not. Neither did cysteamine lower the levels of saposin D, a biomarker found in INCL lymphoblasts.^{43,77} Two analogues of cysteamine, 2-(aminopropyl)aminoethanethiol and dimethylaminoethanethiol, which were reactive in cleaving palmitoyl-CoA, showed elevated levels of saposin D. The authors concluded that cysteamine and its analogues were insufficiently active to produce a therapeutic effect.⁴³ Cysteamine was also tested in four patients with CLN1 mutation in a 7-year open label, nonrandomized trial. Cysteamine showed a decrease in storage material in the patients. However, the reduction in storage material was reversible with decrease or discontinuation of cysteamine. Slower disease progression was observed in two individuals, but the authors could not determine if the slower disease progression was due to the treatment, as slower progression was seen in the same two patients even before treatment began.⁴

Phosphocysteamine (**9**) and *N*-acetylcysteine (**6**) cleave the thioester linkages in [^{14}C]palmitoyl-CoA in a concentration- and time-dependent fashion in vitro. Phosphocysteamine at 0.5 mM concentration disrupted the thioester linkages in S-acetylated proteins in immortalized lymphoblasts of INCL, LINCL, and JNCL patients with PPT1 deficiency. Phosphocysteamine reduced GROD formation after 3 weeks of treatment in INCL patient fibroblasts and lymphoblasts; however, withdrawal of phosphocysteamine led to reaccumulation of ceroid deposits. Phosphocysteamine also reduced levels of saposin A and D in INCL patient lymphoblasts;⁷⁷ elevated levels of saposin A and D are observed in PPT1-deficient INCL cells.⁷⁸ INCL patient lymphoblasts were rescued from apoptosis by treatment with phosphocysteamine.⁷⁷ Phosphocysteamine, an analogue of cysteamine, was used in this study because phosphocysteamine retains the pharmacological properties of cysteamine and does not have a disagreeable odor, unlike cysteamine.

Oxidative stress in neurological disorders can lead to downregulation of antioxidant enzymes such as superoxide dismutase 1 (SOD-1).⁷⁹ Experiments performed in post-mortem brain tissues from INCL patients showed augmented levels of glucose regulated protein 78 (GRP78/BiP) due to elevated ER stress and cleaved poly ADP-ribose polymerase (PARP), indicating apoptosis.^{79,80} Resveratrol (**10**), a potent antioxidant found in grapes, amplified SOD-1 expression in NCL patient lymphoblasts along with reduction in the ER stress marker protein GRP78/BiP and apoptosis marker proteins apoptosis-inducing factor (AIF), cytochrome *c*, and cleaved PARP.⁷⁹ In 6-month-old Ppt1-knockout mice reduced levels of claudin 5, occludin, junctional adhesion molecule 1 (JAM1), and claudin-1 indicated that BBB integrity was breached. Elevated levels of IL-17A-positive T_H17 lymphocytes, observed in the spleen and brain of Ppt1-knockout mice, activate metalloproteinases (MMPs) which are responsible for disruption of the BBB. Elevated levels of MMP-2, MMP-3, and MMP-9 proteins were observed in both Ppt1 knockout (KO) mice brain and in post-mortem brain tissues of INCL patients. Ppt1-KO mice on a resveratrol diet showed reduced levels of IL-17A, MMP-2, MMP-3, and MMP-9 and elevated levels of occludin and claudin 5. Resveratrol suppressed differentiation of T_H17 lymphocytes from naive CD4⁺ T cells isolated from the spleen of Ppt1-KO mice in a dose- and time-dependent manner.⁸¹

PTC124 (Ataluren) (**11**) is currently under clinical trials for diseases such as cystic fibrosis and Duchenne muscular dystrophy, all caused by nonsense mutations.^{82,83} Compound **11** causes ribosomal read-through of premature stop codons and is ineffective against normal stop codons. In patient derived INCL fibroblasts, **11** induced PPT1 enzyme activity in a dose- and time-dependent fashion. PPT1 activity was restored to that of 1.3% of normal fibroblasts. Compound **11** was not toxic in INCL patient fibroblasts unlike gentamicin (**13**). Low levels of PPT1 induced by **11** decreased ceroid deposition and GRODs and protected INCL patient fibroblasts from apoptosis.⁸⁴ Compound **11** significantly elevated TPP1 protein and enzyme levels in CLN2 patient NPCs and showed a reduction in deposition of subunit c of mitochondrial ATP synthase.⁴⁸

Pharmacological chaperones have shown a beneficial effect in lysosomal storage disorders resulting from mis-sense mutation and misfolded proteins.^{85–87} The use of several pharmacological chaperones has been reported in NCL, resulting in the restoration of 2-fold PPT1 activity in INCL patient lymphoblasts with residual enzyme activity but with no observable effect in lymphoblasts with inactive enzyme.⁸⁸ However, only patients with specific types of mutations can be treated with chaperones, limiting this therapy to a subset of patients.⁸⁹

SMALL MOLECULE THERAPEUTICS EVALUATED IN CLN2 DISEASE (LINCL)

A single case has been reported wherein a 5-year-old boy was diagnosed with Jansky–Bielschowsky disease (LINCL), caused by TPP1 deficiency.⁹ 9-Tetrahydrocannabinol (**12**) showed a “considerable improvement” in the child’s condition.⁹⁰ No further details have been reported to date.

12 Tetrahydrocannabinol

13 Gentamicin

Aminoglycoside antibiotics like gentamicin (**13**) can promote read-through of premature stop codons and can be beneficial in disorders caused by nonsense mutations such as cystic fibrosis.⁹¹ Premature stop codon mutations are observed in approximately half of the children diagnosed with LINCL⁹² and in 31% of children diagnosed with INCL.⁸⁴ Treatment with gentamicin showed approximately 5% and 7% increase in TPP1 levels in

cell lines with heterozygous Arg208Stop and heterozygous Arg127Stop mutations, respectively, while it had no effect on a stop codon at Gln66.⁹²

14 Gemfibrozil**15** Fenofibrate

Mutation of the CLN2 gene leads to defective TPP1 causing LINCL. However, a few copies of the normal gene are expressed in patients, supported by the hypothesis that residual TPP1 activity is present.⁹³ The FDA approved lipid lowering drugs gemfibrozil (**14**) and fenofibrate (**15**) upregulate TPP1 mRNA and protein in mouse brain cells, such as primary astrocytes, cortex, hippocampus, and striatum neurons, and in human astrocytes and the SH-SY5Y cell line. The fibrate drugs mediated upregulation of TPP1 via the activation of peroxisome proliferator-activated receptor α /retinoid X receptor α (PPAR α /RXR α) heterodimer as observed in mouse astrocytes and in vivo in mice. PPAR β and PPAR γ were not involved in TPP1 upregulation.⁹⁴ It should be noted, however, that these experiments were performed in mice brain cells without the phenotype for LINCL, translating these observations into patients remains to be studied. Gemfibrozil and fenofibrate failed to increase TPP1 levels in CLN2 patient NPCs, the stem cell model discussed earlier.⁴⁸

SMALL MOLECULE THERAPEUTICS EVALUATED IN CLN6 DISEASE (KUF5 DISEASE TYPE A)

Minocycline (**16**), an antibiotic belonging to the tetracycline family, has been shown to have a beneficial effect on neuroinflammation.⁹⁵ However, in South Hampshire sheep with ovine CLN6 disorder, treatment with minocycline had no preventive effect on the development of blindness, the ratio of gross brain atrophy was unaffected, and microglial and astrocytic activation were not inhibited. The authors concluded that treatment with minocycline does not target chronic inflammation.⁹⁶

16 Minocycline

SMALL MOLECULE THERAPEUTICS EVALUATED IN CLN8 DISEASE

A motor neuron disease (mnd) mouse model, similar to a CLN8 gene mutation in humans, shows AMPA receptor mediated excitotoxicity and accumulation of lysosomal storage material.^{97,98} Chronic treatment with a selective AMPA receptor antagonist **17** 6-nitro-7-sulfamoylbenzo(*F*)quinoxaline-2,3-dione (NBQX) showed an improvement in a range of different tests of motor performance.^{97,99}

17 NBQX **18** ZK 187638

Dosing of 70 mg/kg, but not 140 mg/kg, of the noncompetitive AMPA receptor antagonist **18** (ZK 187638) in mnd mice showed significant enhancement in motor neuron function when assessed by different semiquantitative and quantitative neurological evaluation tests as compared to mnd mice with no treatment. Compound **18** showed 3-fold elevated brain concentration as compared to plasma concentration.¹⁰⁰

The β_2 agonist clenbuterol (**19**) has previously been shown to be neuroprotective.¹⁰¹ Treatment with clenbuterol significantly delayed motor neuron dysfunction in mnd mice as compared to control when assessed by behavioral tests.¹⁰² Clenbuterol treated mnd mice showed significantly reduced eccentrically nucleated motor neurons as compared to untreated mnd mice.¹⁰³

19 Clenbuterol

CONCLUSIONS

Small molecule therapeutics, both experimental and clinically approved, demonstrate potential for the eventual development of a disease-modifying therapy for the treatment of the NCLs (Table 1). Several therapies such as enzyme replacement therapy, gene therapy, and stem cell based therapy have been developed for CLN1 and CLN2 diseases and have reached clinical trial. However, the advantage of small molecules is that they can penetrate the BBB while macromolecules cannot. While the BBB is disrupted in several neurological disorders including Batten disease, normal function would be expected to be restored due to the effect of the therapeutic agent, which may make macromolecules less effective over time.¹⁰⁴ Although the exact pathophysiology that drives the disease is still largely unknown, a variety of targets have emerged that provide hope for drug discovery and development. Active compounds exhibit mechanisms of action including enzyme mimics and upregulators, Bcl-2 upregulators, NMDA and AMPA receptor antagonists, calcium channel blockers, immunosuppressants, antioxidants, pharmacological chaperones, and compounds that promote the read-through of premature stop codons. Many of these modes of action are common across all neurodegenerative diseases, and lessons learned from the study of adult

diseases will transfer to the development of compounds to treat the NCLs with the hope of speeding the process to fruition by learning from history.

Many of the compounds under evaluation for efficacy in the NCLs are clinically approved drugs for other indications such as the analgesic flupirtine, the Alzheimer's disease drug memantine, the immunosuppressant mycophenolate mofetil, and the cholesterol lowering drugs gemfibrozil and fenofibrate. A robust drug repurposing program may allow the rapid advancement of these compounds to clinical trial and allow patients access to the latest research in a much decreased time span.¹⁰⁵

Of equal importance are the hit structures that these compounds represent for further drug discovery efforts. While the initial compounds may prove ineffective due to a high dosage requirement or manifestation of undesirable side effects, medicinal chemistry lead optimization programs are underway in our lab and others to enhance the activity of these compounds while simultaneously removing the undesirable side effects.

While the community awaits the results of the phase II clinical trial of mycophenolate mofetil, research continues on the polypharmacology of the use of two or more agents in synergy, a tactic developed for the treatment of adult neurodegenerative diseases.¹⁰⁶ This approach may well result in measurable improvements in the condition of NCL patients; the combined effects of two or more drugs delivered in synergy are often more efficacious than the individual drugs.

While the NCLs are an orphan disease and lack the investment of more prolific diseases such as cancer, the academic community has embraced the challenge to understand the pathophysiology of the disease, develop robust animal and cellular models and has begun the process of designing and evaluating potential small molecule therapeutics for the treatment of this devastating disease.

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Biographies

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Paul C. Trippier received his D.Phil in Organic Chemistry from Merton College, University of Oxford, U.K., under the supervision of Mark G. Moloney. His research concerned the total synthesis of the natural product oxazolomycin. He carried out postdoctoral research at Cardiff University, U.K., with Chris McGuigan and at Northwestern University, IL, with Richard B. Silverman. At Northwestern his research involved the design, synthesis, and

mode of action determination of small molecules active in murine models of amyotrophic lateral sclerosis. Since 2012 Dr. Trippier has been an Assistant Professor at the School of Pharmacy of TTUHSC and a member of the Center for Chemical Biology where his research focuses on small molecule drug discovery for cancer and neurodegenerative diseases.

ABBREVIATIONS USED

AIF	apoptosis-inducing factor
AMPA	α -amino-3-hydroxy-5-methyl-4-isooxazolepropionic acid
BBB	blood–brain barrier
CSPα	cysteine-string protein α
CPS1	carbamoyl phosphate synthetase 1
ER	endoplasmic reticulum
GROD	granular osmiophilic deposit
INCL	infantile neuronal ceroid lipofuscinosis
iPSC	induced pluripotent stem cell
JAM1	junctional adhesion molecule 1
JNCL	juvenile neuronal ceroid lipofuscinosis
KO	knockout
LINCL	late infantile neuronal ceroid lipofuscinosis
MMP	matrix metalloproteinase
mnd	motor neuron disease
NBQX	6-nitro-7-sulfamoylbenzo-(<i>F</i>)quinoxaline-2,3-dione
NCL	neuronal ceroid lipofuscinosis
NMDA	<i>N</i> -methyl-D-aspartate
NO	nitric oxide
NPC	neural progenitor cell
NtBuHA	<i>N</i> -(<i>tert</i> -butyl)hydroxylamine
OGG1	8-oxoguanine DNA glycosylase 1
PARP	poly ADP-ribose polymerase
PPAR	peroxisome proliferator-activated receptor
PPT1	palmitoyl protein thioesterase 1
RT-PCR	reverse transcription polymerase chain reaction
RXR	retinoid X receptor

SAR	structure–activity relationship
SOD-1	superoxide dismutase 1
TPP1	tripeptidyl peptidase I
UBDRS	unified Batten disease rating scale

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Table 1

Summary of Mechanism of Action and Preclinical Testing of Candidate Therapeutics for NCLs

Structure	Mechanism of Action	Comments	Current Status	References
1. Flupirtine	NMDA receptor antagonist, upregulation of Bcl-2. Other mechanisms possible	Active in in vitro models including CLN1, CLN2, CLN3 and CLN6 patient cells	Survey based study performed in patients was inconclusive. No in vivo experiments performed	34, 59, 60
2. Amlodipine	L-type Calcium channel blocker	Active in in vitro models including patient cells	No follow up studies of amlodipine in vivo. No follow up studies of other calcium channel blockers	47, 62
3. EGIS-8332	AMPA receptor antagonist	Active in vivo in CLN3 ^{ex1-6} mouse model	No follow up study in clinical trials of any AMPA receptor antagonist	63, 64
4. Memantine	NMDA receptor antagonist	Active in vivo in CLN3 ^{ex1-6} and Ppt1 ^{-/-} mice models but further experiments are required. May be useful in INCL patients who are symptomatic	No follow up studies in other in vivo models or in humans	24, 70
5. Mycophenolate mofetil	Immunosuppressant acting by inhibiting inosine monophosphate dehydrogenase	Active in vivo in CLN3 ^{-/-} mouse model	Currently in Phase II clinical trials	71, 72
6. N-acetylcysteine	Antioxidant	Active in in vitro models including patient cells	Pilot study in patients showed a beneficial effect. More studies required	5, 45

Structure	Mechanism of Action	Comments	Current Status	References
7. N-tert-butyl hydroxylamine	Mimics PPT1 enzyme	Active in in vitro models including INCL patient cells as well as in vivo Ppt1 ^{-/-} mouse model	No follow-up studies	44
8. Cysteamine	Mimics PPT1 enzyme	Active in in vitro models including INCL patient cells	Inconclusive results when tested in 4 INCL patients	4, 43, 77
9. Phosphocysteamine	Mimics PPT1 enzyme	Active in in vitro models including INCL patient cells	No follow-up studies	77, 78
10. Resveratrol	Antioxidant	Active in in vitro models including patient cells as well as in vivo mouse model	No clinical trials performed	79, 81
11. PTC124 (Ataluren)	Promotes read through of premature stop codon	Active in vitro in CLN2 stem cell model and INCL patient cells	No follow up studies	84
12. Tetrahydrocannabinol	Unknown	Beneficial effect in a single LINCL patient	No other studies performed	90
13. Gentamicin	Promotes read through of premature stop codon	Active in vitro in LINCL patient cells	No in vivo experiments performed	92
14. Gemfibrozil	Upregulates TPP1 mRNA via PPARα/RXRα	Active in vitro; however no experiments conducted in phenotypic model. No effect in stem cell derived model	No follow-up studies	48, 94

Structure	Mechanism of Action	Comments	Current Status	References
15. Fenofibrate	Upregulates TPP1 mRNA via PPAR α /RXR α	Active in vitro; however no experiments conducted in phenotypic model. No effect in stem cell derived model	No follow-up studies	48, 94
16. Minocycline	Unknown	No effect in vivo in CLN6 sheep model	No follow-up studies	96
17. NBQX	AMPA receptor antagonist	Active in vivo in CLN8 mouse model	No follow-up studies	97, 99
18. ZK 187638	AMPA receptor antagonist	Active in vivo in mnd mouse model	No follow-up studies	100
19. Clenbuterol	Known β 2 agonist but probably not acting by this mechanism in NCL	Active in vivo in mnd mouse model	No follow-up studies	103
Lithium	IMPase inhibition	Active in vitro in phenotypic CLN3 mutant knock-in cerebellar cells	No follow-up studies	74
Pharmacological Chaperones	Stabilize three dimensional conformation of misfolded protein	Active in vitro in INCL patient cells	No follow-up studies	88