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Considerations for the Treatment of Infantile Neuronal Ceroid Lipofuscinosis (Infantile Batten Disease)

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Abstract

The infantile form of neuronal ceroid lipofuscinosis (ie, infantile Batten disease) is the most rapidly progressing type and is caused by an inherited deficiency in the lysosomal enzyme palmitoyl protein thioesterase 1. The absence of enzyme activity leads to progressive accumulation of autofluorescent material in many cell types, particularly neurons of the central nervous system. Clinical signs of infantile neuronal ceroid lipofuscinosis appear between 6 months and 1 year of age and include vision loss, cognitive decline, motor deficits, seizures, and premature death, typically by 3 to 5 years of age. There is currently no effective treatment. However, preclinical experiments in the murine model of infantile neuronal ceroid lipofuscinosis have shown that gene therapy, enzyme replacement, stem cell transplantation, and small molecule drugs, alone or in combination, can significantly slow disease progression. A more thorough understanding of the underlying pathogenesis of infantile neuronal ceroid lipofuscinosis will identify new therapeutic targets.

Keywords

Batten disease; enzyme replacement therapy; gene therapy; infantile Batten disease; lysosomal storage disease; stem cells

Introduction

The neuronal ceroid lipofuscinoses (Batten disease) comprise a class of inherited neurodegenerative disorders typically affecting children.¹ At least 9 different genes are known to cause various forms of neuronal ceroid lipofuscinosis. Most are inherited as autosomal recessive diseases except for the adult-onset dominant form (Parry's disease).^{2,3} The hallmark of the neuronal ceroid lipofuscinoses is progressive accumulation of autofluorescent lipopigment (lipofuscin) in many cells of the body, particularly in neurons of the central nervous system. Clinical signs vary, but most forms present with vision loss, cognitive decline, or motor deficits, and lead to premature death.

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Author Contributions

Mark S. Sands is solely responsible for the contents of this manuscript.

Declaration of Conflicting Interests

Mark S. Sands has no financial conflicts associated with this work.

Ethical Approval

Not applicable

The infantile form of neuronal ceroid lipofuscinosis (infantile Batten disease) is the most rapidly progressing. It is caused by a deficiency in palmitoyl protein thioesterase 1 activity.⁴ Palmitoyl protein thioesterase 1 is a lysosomal enzyme responsible for removing the hydrocarbon chain from palmitoylated membrane-tethered proteins. In the absence of palmitoyl protein thioesterase 1 activity, autofluorescent material accumulates in most cells of the body but is most prominent in neurons of the central nervous system. The ultrastructural nature of the accumulated material is granular osmiophilic deposits, which distinguishes it from other forms of neuronal ceroid lipofuscinosis.¹ Infantile Batten disease is profoundly neurodegenerative; the mass of the brains from affected children is typically only 50% to 60% that of normal age-matched children.⁴ Clinical signs of disease typically appear between 6 months and 1 year of age. However, protracted forms of infantile neuronal ceroid lipofuscinosis have been described that are associated with low levels of palmitoyl protein thioesterase 1 activity. Similar to many of the neuronal ceroid lipofuscinoses, the infantile variant presents clinically with vision loss, cognitive decline, motor deficits, and premature death, typically by 3 to 5 years of age. There is currently no effective therapy for infantile neuronal ceroid lipofuscinosis.

A murine model was developed that is completely deficient in palmitoyl protein thioesterase 1 activity.⁵ The palmitoyl protein thioesterase 1-deficient mouse mimics most of the biochemical and clinical characteristics of the human disease. Autofluorescent material accumulates in most cells of the body, including neurons of the central nervous system, and has the ultrastructural character of granular osmiophilic deposits. The mouse model of infantile neuronal ceroid lipofuscinosis has profound neurodegeneration and cortical atrophy.^{6,7} The mass of the brains from mice with end-stage disease is only 60 to 70% normal. Clinically, the palmitoyl protein thioesterase 1-deficient mouse shares many of the same features as children with infantile neuronal ceroid lipofuscinosis, including motor deficits measured on the rotarod apparatus, vision loss measured by electroretinography, and spontaneous seizures measured by video monitoring and electroencephalography.^{5,8,9}

The palmitoyl protein thioesterase 1-deficient mouse has proved to be a powerful tool to develop and test various therapeutic approaches for infantile neuronal ceroid lipofuscinosis. Gene therapy, enzyme replacement therapy, stem cell transplantation, and small-molecule drugs have demonstrated varying degrees of efficacy in the infantile neuronal ceroid lipofuscinosis mouse (Table 1). Several combinations of the above approaches have resulted in the greatest increase in efficacy. Interestingly, when multiple therapeutic approaches are combined, the effects appear to be synergistic rather than simply additive. As our understanding of the underlying pathogenesis of infantile neuronal ceroid lipofuscinosis increases, additional therapeutic targets will be identified for this invariably fatal neurodegenerative disease.¹⁰⁻¹² In this review/perspective, we discuss the current state of research into treatments for infantile neuronal ceroid lipofuscinosis and describe considerations for increasing efficacy, such as timing of treatment, primary and secondary targets, and systemic vs. central nervous system-specific therapies.

Potential Therapies for Infantile Neuronal Ceroid Lipofuscinosis

Enzyme Replacement Therapy

Infantile neuronal ceroid lipofuscinosis is considered a classic lysosomal storage disease since it is caused by the deficiency of a soluble lysosomal enzyme (palmitoyl protein thioesterase 1).⁴ For this reason, infantile neuronal ceroid lipofuscinosis might be responsive to enzyme replacement therapy with recombinant palmitoyl protein thioesterase 1. Intravenous injection of a recombinant lysosomal enzyme or injection directly into the central nervous system has been shown to reduce accumulation of storage material and improve clinical measures in several lysosomal storage diseases.¹³

Although lysosomal enzymes carry out their function in a subcellular, membrane-bound organelle (the lysosome), a small percentage of these enzymes are secreted from the cell. Mammalian cells have evolved mechanisms to recapture the secreted enzymes and direct them to the lysosome, where they retain their function. This secretion/recapture mechanism was originally referred to as “cross correction” and provided the conceptual framework for enzyme replacement therapy.¹⁴ Several uptake pathways have been identified that involve receptor-mediated endocytosis (mannose and mannose-6-phosphate receptors) and receptor-independent mechanisms (likely fluid-phase pinocytosis).¹⁵ The mannose-6-phosphate receptor is present on most cells of the body and mediates efficient uptake of recombinant lysosomal enzymes that have a mannose-6-phosphate moiety at the terminal end of the polysaccharides that decorate the enzymes.

Intravenous injection of recombinant lysosomal enzymes that have mannose-6-phosphate residues on them has resulted in biochemical and clinical improvements in several lysosomal storage diseases.¹³ Unfortunately, most recombinant lysosomal enzymes will not cross the blood-brain barrier. Therefore, enzyme replacement therapy has been most effective for treating the systemic disease associated with lysosomal storage diseases. However, it has been shown that therapeutic levels of enzyme can be delivered to the central nervous system if very high doses are intravenously injected into newborn or adult animals.¹⁶

Properly modified recombinant palmitoyl protein thioesterase 1 has been produced that is rapidly endocytosed by cultured cells in a mannose-6-phosphate-dependent manner.¹⁷ A preclinical study showed that intravenous injection of this enzyme could significantly reduce the accumulation of storage material, increase the life span, and improve motor function in the infantile neuronal ceroid lipofuscinosis mouse.¹⁸ Interestingly, treatment efficacy was dependent on when treatment was initiated. If the recombinant enzyme was injected into young adult animals, the only improvement was in a reduction in storage material in systemic tissues, with no increase in life span or improvement in motor function. In contrast, if recombinant palmitoyl protein thioesterase 1 was injected intravenously starting at birth, there was a transient reduction of storage material in the central nervous system, reduced neuroinflammation, a persistent reduction of systemic storage material, increased life span, and improved motor function. Although systemic enzyme replacement therapy initiated in the newborn period was more effective than delayed treatment, this approach simply slowed the progression of the disease. The difference in efficacy between early (newborn) and delayed (young adult) treatment is not surprising, since infantile neuronal ceroid lipofuscinosis is a progressive disease. It has been shown previously in another lysosomal storage disease that enzyme replacement therapy initiated during the newborn period is more effective than delaying treatment.¹⁹

Although this approach was only partially effective, even when initiated at birth, it demonstrates that delivery of recombinant palmitoyl protein thioesterase 1 can reduce storage material and improve the clinical outcome. Injection of recombinant palmitoyl protein thioesterase 1 directly into the central nervous system might further increase the efficacy of enzyme replacement therapy for infantile neuronal ceroid lipofuscinosis. This has been demonstrated in the murine model of late-infantile neuronal ceroid lipofuscinosis.²⁰ Intrathecal injection of tripeptidyl peptidase 1, the enzyme deficient in late-infantile neuronal ceroid lipofuscinosis, resulted in much greater clinical benefit compared with intravenous injection of enzyme. Similar results have been obtained in several other models of lysosomal storage disease after intrathecal injection of enzyme.²¹ Alternatively, intravenous injection of recombinant palmitoyl protein thioesterase 1 could be used as one component of a more comprehensive therapeutic regimen where multiple approaches are combined.

Gene Therapy

The fundamental idea behind gene therapy for lysosomal storage diseases is that a functional copy of the defective gene can be stably introduced into a cell via a vector such that the diseased cell can synthesize its own enzyme.²² This is typically accomplished by taking advantage of the mechanisms viruses have evolved to efficiently transfer (transduce) their genetic material into a host cell. Briefly, the genes responsible for viral replication and disease are removed from the recombinant virus and replaced by a functional copy of the defective gene. The recombinant vector genome is then packaged into a viral particle that can be used to transduce affected cells. In this way, the recombinant viral vector can transfer a functional copy of the defective gene into a cell but it is incapable of replicating and producing infectious viral particles. Numerous viruses, including adenoviruses, adeno-associated viruses, and human immunodeficiency virus, among others, have been engineered for this purpose.

Gene therapy approaches rely on the same fundamental principle of ‘cross-correction’ as enzyme replacement therapy. The viral gene therapy vectors currently available are not able to transfer a functional copy of the defective gene to all the cells of the central nervous system. Consequently, in this approach a minority of the cells in the central nervous system are transduced. However, viral gene transfer vectors are typically engineered to over-express the deficient enzyme in that small population of cells. It has been shown that transduced cells that overexpress a lysosomal enzyme also secrete a proportionately high level of enzyme.²³ In principle, the higher level of secreted enzyme could “cross correct” a relatively large sphere of untransduced cells. Therefore, gene therapy can be viewed as a form of enzyme replacement therapy. The major differences are that classic enzyme replacement therapy is transient in nature and the enzyme comes from an exogenous source. In contrast, gene therapy has the potential to provide a continuous supply of enzyme from an endogenous source.

Gene therapy approaches can be grouped into 2 general categories: (1) in vivo gene therapy and (2) ex vivo gene therapy. In vivo gene therapy refers to the administration of the recombinant viral vector directly to the patient. With respect to a neurodegenerative disease like infantile neuronal ceroid lipofuscinosis, a viral gene transfer vector could be injected into the brain parenchyma intracranially, intrathecally, intracerebroventricularly, or intraocularly. Examples of viral vectors that might be used for this approach are adenovirus, adeno-associated virus, and lentivirus. Ex vivo gene therapy refers to the genetic modification of the patient’s own cells or donor cells outside the body and then transplantation of those cells back into the affected patient. This is most commonly thought of in reference to hematopoietic stem cells. However, it is relatively easy to imagine a similar approach using neuronal stem cells or mesenchymal stem cells. The most common viral gene transfer vectors used for this approach are the retroviral vectors (murine-based retroviruses and lentiviruses), since they stably integrate into the host genome and will not be lost as the cells divide.

To date, no gene therapy approach has been attempted in a patient with infantile neuronal ceroid lipofuscinosis. However, central nervous system-directed gene therapy using an AAV2 vector has been shown to be safe in children with late-infantile neuronal ceroid lipofuscinosis.²⁴ In addition, numerous gene therapy experiments have been performed in various models of lysosomal storage disease, including the mouse model of infantile neuronal ceroid lipofuscinosis.²⁵ Direct intracranial injection of a first-generation AAV vector (AAV2) into newborn infantile neuronal ceroid lipofuscinosis mice resulted in persistent localized expression of palmitoyl protein thioesterase 1 near the injection site.^{7,9} Although this approach resulted in reduced lysosomal storage, it had no effect on life span or motor function. However, the identical approach using a second-generation AAV vector

pseudotyped with the AAV5 capsid protein (AAV2/5) resulted in a 50% increase in life span and corresponding improvement in motor function.²⁶ Injection of an AAV2 vector into the vitreous of the eye resulted in stable palmitoyl protein thioesterase 1 expression in the retinal ganglion cells and partial correction of visual defects as measured by electroretinography.⁸ Interestingly, palmitoyl protein thioesterase 1 activity could also be detected in the optic nerve, lateral geniculate nucleus, and superior colliculus. The palmitoyl protein thioesterase 1 activity that was delivered to the brain by anterograde axonal transport was sufficient to reduce neurodegeneration throughout the visual tracts of the infantile neuronal ceroid lipofuscinosis mouse.⁸

Although central nervous system-directed gene therapy was only partially effective, the development of newer generation vectors such as AAV2/9, which is reported to cross the blood-brain barrier, might dramatically improve efficacy. For example, intravenous delivery of an AAV2/9 gene transfer vector into mouse fetuses resulted in widespread expression throughout the brain, peripheral nervous system, and systemic tissues.²⁷ Alternatively, as our understanding of the disease pathogenesis increases, more precise targeting of affected brain regions will likely increase efficacy.

Stem Cell Transplantation

Transplantation of stem cells into the brains of an affected patient is another approach that holds promise for the treatment of infantile neuronal ceroid lipofuscinosis. In this particular case the stem cells, depending on their origin, could serve one or more of the following functions: (1) act as a source of the deficient enzyme, (2) provide trophic support, (3) have anti-inflammatory effects, and (4) replace damaged or dead cells. The choice of stem cells rather than fully differentiated cells also has the potential to provide a long-lasting therapeutic effect. Fully differentiated cells have a finite life span and any therapeutic effect will wane as those cells are gradually lost. In contrast, stem cells have the potential for self-renewal and many rounds of division, thereby raising the possibility for a persistent, perhaps lifelong therapeutic effect.

Numerous stem cell sources could be considered for infantile neuronal ceroid lipofuscinosis. Given that infantile neuronal ceroid lipofuscinosis is primarily a neurodegenerative disorder, neuronal stem cells would seem an obvious candidate. Therefore, the efficacy of transplanting normal human-derived neuronal stem cells into the brains was determined in a xenotransplant model of infantile neuronal ceroid lipofuscinosis (*Ppt1*^{-/-}/NODSCID mice).²⁸ The human neuronal stem cells secreted palmitoyl protein thioesterase 1 capable of being endocytosed in a mannose-6-phosphate-dependent manner. The neuronal stem cells migrated throughout the neuroaxis, persisted for at least 4 months, reduced autofluorescent accumulation, and improved motor function. However, it is difficult to evaluate the longterm effects of the neuronal stem cells due to one of the major limitations of the xenotransplant model, a short life span. Although the short-term data look promising, it is unlikely that the normal neuronal stem cells completely corrected the disease. More complete correction might have been achievable if the neuronal stem cells had been transduced with a gene transfer vector to overexpress palmitoyl protein thioesterase1. Another reason to explore the use of neuronal stem cells is that they might have the potential to replace dead and dying neurons. However, infantile neuronal ceroid lipofuscinosis is a profoundly neurodegenerative disease that progresses rapidly. Therefore, it seems unlikely that transplanted neuronal stem cells could replace the vast number of dead neurons and faithfully recapitulate the proper neuronal connections. A human clinical trial using neuronal stem cells was performed in children with infantile neuronal ceroid lipofuscinosis. The results of that study have yet to be published.

Another source of stem cells that has been used to treat lysosomal storage diseases is hematopoietic stem cells.²⁹ The principle behind using hematopoietic stem cells is that the patient's entire hematopoietic system can be replaced by donor cells that express normal levels of the deficient lysosomal enzyme. As the normal donor-derived cells (typically from bone marrow or cord blood samples) circulate throughout the body, they will secrete lysosomal enzymes that can cross-correct surrounding tissues. In this way the hematopoietic system serves as an enzyme delivery system to most tissues of the body. In addition to supplying enzyme, hematopoietic stem cell transplantation has been shown to provide anti-inflammatory functions.³⁰⁻³² This could be particularly important in infantile neuronal ceroid lipofuscinosis, since one of the first histological signs of disease is neuroinflammation. Unfortunately, bone marrow transplantation has proved ineffective in these children.³³ This is consistent with outcomes observed in the murine model of infantile neuronal ceroid lipofuscinosis, where bone marrow transplantation had no effect on disease progression.³⁴ In general, bone marrow transplantation can be modestly effective for the systemic disease associated with lysosomal storage diseases but is largely ineffective for the central nervous system component of these disorders. The notable exception is Krabbe disease, where bone marrow transplantation has been shown to be partially effective.³⁵

As mentioned above, neuroinflammation is one of the first histological signs of disease in the palmitoyl protein thioesterase 1-deficient mouse.¹⁰ The neuroinflammation is initially localized to the thalamus and then spreads throughout the neuroaxis as the disease progresses. It has been shown previously that bone marrow- and adipose-derived mesenchymal stem cells can mediate anti-inflammatory effects. Krabbe mice receiving intracerebroventricular injections of mesenchymal stem cells had a small but significant increase in life span, body weight, and improved motor function.³⁶ Interestingly, there was also a decrease in macrophage infiltration and numerous pro-inflammatory markers. Mesenchymal stem cells could also be used as a source of enzyme since they can be efficiently transduced, express high levels of protein, migrate throughout the body, and persist for many months in vivo.³⁷ To date, mesenchymal stem cells have not been explored as a potential treatment in either the palmitoyl protein thioesterase 1-deficient mouse or children with infantile neuronal ceroid lipofuscinosis.

Small-Molecule Drugs

As the underlying pathogenesis of infantile neuronal ceroid lipofuscinosis is more thoroughly understood, additional therapeutic targets are being discovered, some of which can be approached with small-molecule drugs. For example, the primary defect in infantile neuronal ceroid lipofuscinosis is a lack of palmitoyl protein thioesterase 1 activity, which removes palmitate from membrane-anchored palmitoylated proteins. Small-molecule drugs that attack the thioester linkage between the protein and palmitate residue, palmitoyl protein thioesterase 1 mimetics, might be able to carry out a similar function as palmitoyl protein thioesterase 1. In fact, the small-molecule lysosomotropic drug phosphocysteamine (Cystagon) was shown to cleave thioester bonds in vitro and reduce the accumulation of storage material and apoptosis in cultured infantile neuronal ceroid lipofuscinosis lymphoblasts.³⁸ A clinical trial was performed in children with infantile neuronal ceroid lipofuscinosis but the results have yet to be published. However, a preclinical study in the palmitoyl protein thioesterase 1-deficient mouse showed that intraperitoneal injections of Cystagon by itself had little or no effect on disease progression.²⁶ Although these data suggest that Cystagon will be ineffective by itself, it may provide benefit as an adjunct to other forms of therapy (see below).

Since infantile neuronal ceroid lipofuscinosis is an inherited disease and many of the patients have nonsense mutations leading to premature translation termination,³⁹ treatment with aminoglycosides might increase palmitoyl protein thioesterase 1 levels to therapeutic or

near-therapeutic levels. The aminoglycoside antibiotic gentamycin can suppress premature termination caused by nonsense mutations. Unfortunately, chronic exposure to gentamycin is associated with significant toxicity. An alternate drug, PTC124, was developed that has stop codon read-through activity but was less toxic than gentamycin.^{40,41} PTC124 was shown to increase palmitoyl protein thioesterase 1 activity to greater than 1% normal and decrease storage material in cultured infantile neuronal ceroid lipofuscinosis fibroblasts harboring a nonsense mutation.⁴² Importantly, there was little or no toxicity observed at the effective drug concentrations. Although 1% normal levels of palmitoyl protein thioesterase 1 activity is still low, it is generally believed that low levels of lysosomal enzymes (1% to 5% normal) can provide some therapeutic benefit. As with a palmitoyl protein thioesterase 1 mimetic, a stop codon read-through drug may have its greatest benefit in combination with other therapies. It will be important to test this class of drug in an animal model harboring a nonsense mutation in the *PPT1* gene. Unfortunately, this model has yet to be developed.

Certain missense mutations can cause proteins to be improperly folded. Those improperly folded proteins are often rapidly degraded in the endoplasmic reticulum by a mechanism referred to as the unfolded protein response.⁴³ It has been shown that competitive inhibitors of certain lysosomal enzymes can stabilize the nascent protein and restore partial activity.¹³ Several mutations in the *PPT1* gene have been identified that appear to destabilize the enzyme.⁴⁴ Non-hydrolyzable competitive inhibitors of palmitoyl protein thioesterase 1 have been shown to be taken up by cultured fibroblasts with missense mutations and increase enzyme activity by as much as 2-fold.⁴⁴ Similar results were obtained in several different cell lines with mutations believed to destabilize palmitoyl protein thioesterase 1. As with stop codon read-through therapy, chaperone therapy by itself may not be able to increase enzyme activity sufficiently to effectively treat infantile neuronal ceroid lipofuscinosis. However, it could enhance other treatment modalities to overcome the therapeutic threshold.

Oxidative stress is a common secondary effect of a variety of neurodegenerative diseases as well as lysosomal storage diseases. Markers of oxidative stress are increased in cultured infantile neuronal ceroid lipofuscinosis fibroblasts and in neurons from palmitoyl protein thioesterase 1-deficient mice.⁴⁵ Exposure of fibroblasts from a patient with infantile neuronal ceroid lipofuscinosis to resveratrol, an antioxidant polyphenol, decreased markers of oxidative stress.⁴⁶ Similarly, markers of oxidative stress were reduced in vivo when palmitoyl protein thioesterase 1-deficient mice were fed a diet containing resveratrol.⁴⁶ Perhaps most importantly, palmitoyl protein thioesterase 1-deficient mice treated with resveratrol had a significant increase in life span. Although the increase in life span was relatively small, ~2 weeks, it is quite impressive considering that resveratrol targets a secondary effect of palmitoyl protein thioesterase 1-deficiency rather than the primary defect. In fact, the increase in life span is greater than that observed in the initial central nervous system-directed gene therapy experiments designed to increase palmitoyl protein thioesterase 1 activity.^{7,9} It is unlikely that resveratrol will provide long-lasting clinical benefit since it does not target the primary defect. However, it could work in concert with other treatments that increase enzyme activity.

Combination Treatments

Lysosomal enzymes, including palmitoyl protein thioesterase 1, are ubiquitously expressed. Consequently, a deficiency in any one of the enzymes can affect many, if not most, cells of the body. Therefore, although lysosomal storage diseases are simple monogenic diseases, they typically have a complicated clinical phenotype. Infantile Batten disease is no exception. Although the most striking and acute feature of infantile neuronal ceroid lipofuscinosis is the central nervous system disease, autofluorescent material accumulates in many tissues of the body. Granular osmiophilic deposits are observed in the liver, spleen,

kidney, pancreas, bone, heart, and eye in the palmitoyl protein thioesterase 1-deficient mouse.⁴⁷ The accumulation of storage material in the heart and great vessels is significant enough to cause aortic dilatation and left ventricular hypertrophy that is detectable by echocardiography.⁴⁷ It seems unlikely that any single therapy will be able to effectively treat all of the tissues affected by palmitoyl protein thioesterase 1-deficiency. For example, if the central nervous system disease is effectively treated (eg, central nervous system-directed gene therapy) then the systemic disease will most likely become clinically relevant. Alternatively, if the systemic disease is eliminated (eg, intravenous enzyme replacement therapy) then the central nervous system disease will continue to progress. Simultaneous systemic- and central nervous system-directed therapies could overcome this hurdle.

Another complication that could be overcome by using a combination of therapies is the timing of treatment. It is possible that an immediate but transient source of enzyme such as systemic or central nervous system-directed enzyme replacement therapy could be used to keep the disease at bay while a more permanent treatment (ie, gene therapy) is being developed or an appropriate donor (bone marrow transplantation or stem cell therapy) is identified. An optimal therapy might also include treatments that target both the primary enzyme deficiency (enzyme replacement therapy or gene therapy) and secondary effects (inflammation, oxidative stress, etc.). Lastly, physical and physiological barriers could be overcome with appropriate combinations of therapy. For example, subtherapeutic levels of enzyme in regions of the brain resulting from the limited diffusion of viral gene transfer vectors and cross-correcting enzyme could be complemented by a small molecule drug that uniformly distributes throughout the brain. Proof-of-principle experiments in the palmitoyl protein thioesterase 1-deficient mouse and other murine models of lysosomal storage diseases have demonstrated the utility of a combination approach.⁴⁸

As mentioned above, central nervous system-directed gene therapy with a second-generation AAV vector (AAV2/5) was moderately effective in the murine model of infantile neuronal ceroid lipofuscinosis.²⁶ However, the disease still progressed in the treated mice and they eventually succumbed to the disease. Therefore, an experiment that combined AAV2/5-mediated gene therapy with the palmitoyl protein thioesterase 1 mimetic phosphocysteamine was performed in the infantile neuronal ceroid lipofuscinosis mouse.²⁶ The hypothesis was that phosphocysteamine, being a small-molecule drug, would be able to penetrate regions of the brain that were not effectively reached by the gene transfer vector or secreted enzyme. Phosphocysteamine alone provided essentially no clinical benefit. However, the combination was more effective than either treatment alone.

In a similar experiment, AAV2/5-mediated central nervous system-directed gene therapy was combined with a small-molecule anti-inflammatory that targets neuroinflammation (Macauley et al., submitted). The anti-inflammatory alone decreased several markers of inflammation but resulted in no increase in life span or improvement in motor function. In contrast, infantile neuronal ceroid lipofuscinosis mice receiving both the anti-inflammatory and central nervous system-directed gene therapy had reduced markers of inflammation, decreased autofluorescent material, and significantly increased life span and motor function compared with those that received either therapy alone.

The most dramatic example of 2 therapies synergizing to increase efficacy is central nervous system-directed gene therapy combined with bone marrow transplantation.³⁴ In this case, AAV2/5-mediated gene therapy alone increased the life span of palmitoyl protein thioesterase 1-deficient mice from ~8 months to ~12 months, similar to the increase reported previously.^{26,34} Bone marrow transplantation alone did not improve any measure of disease (palmitoyl protein thioesterase 1 activity, inflammation, neurodegeneration, life span, etc.). In fact, palmitoyl protein thioesterase 1-deficient mice treated with bone marrow

transplantation alone had impaired motor function compared with untreated infantile neuronal ceroid lipofuscinosis mice. However, when central nervous system-directed gene therapy was combined with bone marrow transplantation, the life span of the combination-treated animals increased to ~18 months.³⁴ There was a corresponding dramatic improvement in motor function in the combination-treated infantile neuronal ceroid lipofuscinosis animals. It is surprising and truly striking that a therapy (bone marrow transplantation) that provides no benefit by any measure would synergize to such an extent with another therapy that provides a modest increase in efficacy. These data suggest that other therapies such as enzyme replacement therapy and resveratrol that result in a small but significant increase in efficacy could synergize with central nervous system-directed gene therapy equally well or better. These data will have to be obtained empirically since it is difficult to predict or model how these various treatments will interact.

Summary

Infantile neuronal ceroid lipofuscinosis is a rapidly progressing inherited neurodegenerative disease caused by a deficiency in the lysosomal enzyme palmitoyl protein thioesterase 1. No effective treatment currently exists for this invariably fatal disorder, which affects children. However, numerous experimental therapeutic approaches have been tested in the mouse model of infantile neuronal ceroid lipofuscinosis. The approaches include central nervous system-directed gene therapy, stem cell transplantation, enzyme replacement therapy, and small-molecule drugs. Varying degrees of correction have been observed with approaches that target the primary defect (enzyme deficiency) and secondary effects (inflammation, oxidative stress, etc.). However, the greatest degree of efficacy was observed with combinations of therapy that target both the primary and secondary defects. As the underlying pathophysiology of infantile neuronal ceroid lipofuscinosis is more completely understood, additional therapeutic targets will be uncovered. As therapies continue to improve, the final hurdle will be identification of affected children when they are pre-symptomatic. This will likely require a newborn screening initiative since the clinical signs of infantile neuronal ceroid lipofuscinosis become apparent at 6 months of age and the disease progresses rapidly from there.

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Table 1**Potential Treatment Strategies for Infantile Neuronal Ceroid Lipofuscinosis**

<u>Treatment</u>	<u>Preclinical</u> [€]	<u>Clinical Trial</u>	<u>Response</u> [£]	<u>Citations</u>
ERT	<i>in vitro</i>	-	↑ PPT1 activity	17
	<i>in vivo</i>	-	B, H, C	18
Gene therapy	<i>in vivo</i> (IC, AAV2)	-	B, H	7,9
	<i>in vivo</i> (IO, AAV2)	-	B, H, C	8
	<i>in vivo</i> (IC, AAV2/5)	-	B, H, C	25
Stem cell therapy	<i>in vivo</i> (NSC)	-	B, H, C	28
	-	NSC	NP	-
	-	HSC	Ø	33
	<i>in vivo</i> (HSC)	-	Ø	34
Small-molecule drug	<i>in vitro</i> (Cystagon)	-	↓ storage material	38
	<i>in vivo</i> (Cystagon)	-	Ø	25
	-	Cystagon	NP	-
	<i>in vivo</i> (resveratrol)	-	B, H, C	46
	<i>in vitro</i> (PTC124)	-	↑ PPT1 activity	42
	<i>in vitro</i> (PPT1 inhibitor)	-	↑ PPT1 activity	44
Combination therapy	<i>in vivo</i> (AAV2/5+Cystagon)	-	B, H, C	25
	<i>in vivo</i> (AAV+BMT)	-	B, H, C	34

AAV2, adeno-associated virus type 2 vector; BMT, bone marrow transplantation; Cystagon, ERT, enzyme replacement therapy; HSC, hematopoietic stem cells; IC, intracranial injection; IO, intraocular; HSC, hematopoietic stem cells; Minozac, antiinflammatory; NSC, neuronal stem cells; Cystagon – palmitoyl protein thioesterase 1 mimetic; resveratrol, antioxidant.

[€] *in vivo*, experiments performed in a murine model of infantile neuronal ceroid lipofuscinosis; *in vitro*, experiments performed in cultured cells.

[£] B, biochemical improvement; C, clinical improvement; H, histological improvement; NP, not published; Ø, no improvement.