ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Review Acta Neurobiol Exp 2008, 68: 429–442

Treatment of lysosomal storage disorders: Focus on the neuronal ceroid-lipofuscinoses

Chris Pierret, Jason A. Morrison, and Mark D. Kirk*

Division of Biological Sciences, University of Missouri, Columbia, MO 65211, USA, *Email: KirkM@missouri.edu

Recent advances in our understanding of lysosomal storage disorders (LSDs) may lead to new therapies to treat the neuronal ceroid-lipofuscinoses (NCLs). In this review, enzyme replacement therapy, gene therapy, cell-mediated therapy and pharmaceutical treatments are considered across the LSDs and extended to therapies for the NCLs. It is likely that a combination of approaches will produce the most beneficial clinical outcome for treatment of pathologies displayed by the NCLs.

Key words: lysosomal storage, neuronal ceroid-lipofuscinoses, enzyme replacement, stem cell, gene therapy, substrate reduction

INTRODUCTION

The neuronal ceroid-lipofuscinoses (NCLs) are a group of neurodegenerative lysosomal storage disorders (LSDs) with an incidence of 1:12 500 live births (Rider and Rider 1988, Haltia 2006, Hobert and Dawson 2006, Jalanko et al. 2006, Kyttala et al. 2006, Siintola et al. 2006a), often referred to collectively as Batten's disease (Zeman and Dyken 1969). To date, age of onset and type of lysosomal storage material are used to define the phenotype of a NCL, though a growing understanding of the genetic basis for the diseases will provide a more definitive characterization based on mutations in identified genes and gene products (Table I) (Haltia 2006, Hobert and Dawson 2006, Jalanko et al. 2006, Siintola et al. 2006a). Animal models for the study of NCLs now include several mouse knockouts and natural mutants that provide essential tools for development of new therapeutic strategies to alleviate the devastating effects of these fatal disorders (Cooper et al. 2006).

Appropriate trafficking of macromolecules to the lysosome is a complex process occurring *via* a number of cellular mechanisms. Once in the lysosome, macromolecules face degradation by more than 50 different acid hydrolases that enter the lysosome by specific trafficking mechanisms (Beck 2007, van Meel and Klumperman 2008). Following protein synthesis, enzymes destined for the lysosome must cross the rough endoplasmic reticulum and be modified for uptake by the lysosome. Most lysosomal enzymes recognize mannose residues, attach to N-acetylglucosamine-1-phosphate, then remove the N-acetylglucosamine residues to expose the mannose-6-phosphate marker (Beck 2007). A common pathway leading to uptake of enzymes by the lysosome includes attachment to the mannose-6-phosphate receptor of the trans-Golgi network, subsequent packing into vesicles coated with clathrin and transport into the late endosome with subsequent lysosomal maturation (Kornfeld and Mellman 1989, Beck 2007, van Meel and Klumperman 2008). Enzymes not bound to the mannose-6-phosphate receptor can be secreted and subsequently uptaken by neighboring cells (Beck 2007). There are also mechanisms for entry into the lysosome that are independent of the mannose-6-phosphate receptor. One such mechanism was described recently to include binding of β-glucocerebrosidase to the lysosomal membrane protein LIMP-2 and transport into the lysosome (Reczek et al. 2007, van Meel and Klumperman 2008).

Lysosomal membrane proteins add additional layers of complexity to the machinery of macromolecule degradation and recycling associated with lysosomal function. These proteins act to separate the acid hydrolases from other incoming molecules, acidify the lysosome,

Correspondence should be addressed to M.D. Kirk, Email: KirkM@missouri.edu

Received 18 December 2007, accepted 8 April 2008

Table I

Classification of human neuronal ceroid-lipofuscinoses

Gene	Gene Product	Product Type	Stored Protein	Onset	Clinical Presentation
CLN1	PPT1 (palmitoyl protein thioesterase 1) (Vesa et al. 1995)	Soluble Enzyme	Sphingolipid activator proteins (SAPs)	Infantile, Late Infantile, Juvenile and Adult	Decreased head growth, retarded psychomotor development, epileptic seizures, muscular hypotonia, ataxia, visual failure, severe brain atrophy
CLN2	TPP1 (tripeptidyl peptidase 1) (Sleat et al. 1997)	Soluble Enzyme	Subunit c of Mitochondrial ATP synthase (SCMAS)	Late infantile, Juvenile and Protracted	Seizures, ataxia, myoclonus, developmental and mental retardation, visual failure, speech impairment
CLN3	CLN3/Battenin (IBDC 1995)	Lysosomal Transmembrane Protein	SCMAS	Juvenile, Adult	Progressive visual failure, blindness, seizures, psychomotor deterioration
CLN4	Unknown	Unknown	SCMAS	Adult	Dementia, progressive myoclonus epilepsy, ataxia, late pyramidal and extra-pyramidal features, behavioral changes, motor disturbances
CLN5	CLN5 (Savukoski et al. 1998)	Lysosomal Glycoprotein (may have soluble form)	SCMAS	Late infantile, Juvenile and Protracted	Similar to other late infantile variants, with slightly later onset
CLN6	CLN6 (Gao et al. 2002, Wheeler et al. 2002)	Transmembrane Protein (ER)	SCMAS	Late infantile	Similar to other late infantile variations, with slightly later onset
CLN7	MFSD8 channel (Siintola et al. 2007)	Membrane Protein	SCMAS	Late infantile (variant)	Seizures, psychomotor decline, myoclonus, loss of vision
CLN8	CLN8 (Ranta et al. 1999)	Transmembrane Protein (ER)	SCMAS	Late infantile (variant)	Epilepsy with progressive mental retardation, seizures, progressive mental deterioration, motor and behavioral problems (Mouse CLN8 -/- shows loss of vision)
CLN9	CLN9 (Schulz et al. 2004)	Unknown	SCMAS	Juvenile	Similar to other juvenile onsets
CTSD	Cathespsin D (Siintola et al. 2006b)	Soluble Enzyme	SAPs	Congenital and Late infantile	Congenital form of NCL
Unknown	Unknown (Siintola et al. 2006a)	Unknown	SAPs	Adult	Parry Disease, similar to other adult onset NCLs

play a role in fusion between the lysosome and other organelles and can transport small molecules into the cytoplasm (Beck 2007). Further processing of metabolites after acid hydrolysis in lysosomes includes translocation back into the cytosol and their use as building blocks for new intracellular products (Beck 2007, van Meel and Klumperman 2008).

Lysosomal storage disorders can result from defects at many points along pathways involved in the trafficking and/or hydrolysis of macromolecules. The types of storage materials that accumulate in the lysosome categorize these disorders. The resultant storage defect may cause an inflammatory response resulting in apoptosis (Kacher and Futerman 2006).

Clinical presentation of LSDs often includes enlargement of the spleen and liver. Related pathologies of the CNS include a wide spectrum of cognitive and motor disorders (Kacher and Futerman 2006, Beck 2007, van Meel and Klumperman 2008), and abnormal function within the CNS often leads to a grave prognosis (Kacher and Futerman 2006). Generally, the level of normal activity remaining in the lysosome dictates the severity of the disorder.

The NCLs manifest themselves in much the same way as other LSDs, but are characterized separately because of their unique lysosomal storage materials and severity of the associated CNS pathologies. All NCLs include build up of acid-Schiff and Sudan Black B-positive lysosomal granules in the cytoplasm of most nerve cells and other cell types. Age of onset and type of lysosomal storage material further define the NCLs (Haltia 2006). Although all cell types show accumulation of storage material in the lysosomes, neurons are selectively lost. Associated with the progressive loss of neurons in the CNS, there is an increased proliferation and hypertrophy of astrocytes. These aspects of NCLs are the focus of current studies in comparative transcriptomics, proteomics, and lipodomics (Jalanko et al. 2006).

Recent genetic characterizations led to re-classification of the NCLs so as to distinguish them from LSDs and other neurodegenerative disorders (Haltia 2006, Hobert and Dawson 2006, Jalanko et al. 2006, Kyttala et al. 2006, Siintola et al. 2006a). However, this has diverted focus from pathological mechanisms that NCLs have in common with LSDs such as Gaucher disease, Tay Sachs, Niemann-Pick, Mucopolysaccharidosis, and Wolman disease. As these diseases all appear to result from abnormal intracellular accumulation of storage materials within vesicles, an analysis of therapeutic strategies used to treat LSDs will likely provide insight into effective treatment strategies for the NCLs. The type of gene product indicative of a particular disease may complicate treatments common among LSDs. For instance, it may prove more difficult to replace the function of transmembrane proteins than soluble enzymes.

Here we review briefly the NCLs including genotypic, proteomic and clinical data (Table I) and describe current therapeutic options for LSDs, highlighting enzyme replacement therapy (ERT), gene therapy, cell-mediated therapies, and pharmacological treatments. LSDs discussed here are categorized in Table II. We suggest a combined therapeutic strategy for the NCLs based on our knowledge of NCL and LSD etiologies and on the most effective individual therapies that have been tested.

ENZYME REPLACEMENT THERAPY (ERT)

Enzyme replacement gained favor for treatment of LSDs after promising clinical trials; intravenous delivery of purified enzymes showed therapeutic activity in Pompe, Fabry, and Gaucher diseases (Desnick et al. 1980, Desnick 2004). Clinical trial NCT00520143 recently resulted in the expanded access of Myozyme for treatment of Pompe Disease (FDA 2006). Approval has also been made for ERT for MPS I along with completed trials for MPS II and VI (Desnick 2004, Piotrowska et al. 2006). However, the challenges of ERT include obtaining a consistent source of purified enzymes, achieving post-translational modifications necessary for trafficking of the enzymes, developing animal models for testing therapeutic actions and overcoming the blood brain barrier (BBB) after systemic enzyme delivery. Other challenges for ERT include costly life-long dosing to maintain a clinical benefit and availability to patients.

The first efforts toward ERT began in the early 1970's (Desnick 2004). In the first clinical trials, Brady and colleagues successfully reduced ganglioside storage in Tay Sachs patients, but did not see clinical recovery (Johnson et al. 1973). Later, having identified β-glucosidase activity as deficient in Gaucher patients, Brady and coauthors (1974) used purified β-glucosidase to attempt ERT. In this trial, it was found that the enzyme was unable to be targeted into macrophages, the site of abnormal lysosomal storage in Gaucher type 1 disease. In subsequent studies, others showed that sequential treatment of β-glucosidase with neuraminidase,

Table II

Classification of human lysosomal storage disorders

Storage Disorder	Protein Defect	Storage Material	CNS Involved?	Clinical Presentation	Citations
α-Mannosidosis	α-mannosidase	Mannose containing oligosaccharides	Yes	Behavior/learning difficulty, skeletal abnormality, immune deficiency, hearing impairment	(Desnick 2004, Crawley et al. 2006, Beck 2007)
Cystinosis	CTNS, cystine transporter	Intracellular cystine crystals	Yes, if untreated	Renal Fanconi Syndrome, renal glomerular failure, systemic complications	(Kleta and Gahl 2004)
Fabry	α-galactosidase	Globotriasylceramide (Gb3)	Yes	Multisystemic (involving heart, kidney and CNS), angiokeratoma	(Desnick 2004, Beck 2007)
Gaucher Type I	β-glucosidase	Glucocerebrosides	No	Hepatosplenomegaly, anemia, thrombocytopenia, skeletal complications	(Desnick 2004, Beck 2007)
Gaucher Type II	β-glucosidase	Glucocerebrosides	Yes	Acute neuropathy, severe visceral, hematological involvement, skeletal involvement, supranuclear gaze palsy, seizures	(Desnick 2004, Beck 2007)
Gaucher Type III	β-glucosidase	Glucocerebrosides	Yes	Chronic neuropathy, continuum of presentation found in Type II	(Desnick 2004, Beck 2007)
Hurler (a form of MPS I)	α-iduronidase	Dermatan sulphate and heparan sulphate	Yes	Skeletal disease, organomegaly, heart disease, mental retardation	(Desnick 2004, Futerman and van Meer 2004, Beck 2007)
Metachromatic Leukodystrophy	Arylsulfatase A	Sulfatide	Yes	Myelin degeneration in CNS and peripheral nervous system, progressive ataxia, seizures, quadriplegia, decerebration	(Desnick 2004, Biffi et al. 2006, Beck 2007)

Mucopolysaccharidosis I (MPS I)	α-iduronidase	Dermatan sulphate and heparan sulphate	In Hurler	See Hurler, may also include corneal clouding, joint contractions	(Desnick 2004, Futerman and van Meer 2004, Beck 2007)
Mucopolysaccharidosis VII	β-glucoronidase	Dermatan sulphate, heparan sulphate, chondroitin-4 and -6 sulphates	Yes	Short stature, mental retardation, hepatosplenomegaly, skeletal deformities, excessive excretion of urinary mucopolysaccharides	(Desnick 2004, Futerman and van Meer 2004, Nakama et al. 2006, Beck 2007)
Niemann-Pick Type C	NPC I / II	Gangliosides (GM2), Cholesterol	Yes	Diverse: neonatal hepatitis, cerebellar ataxia, cognitive impairment, supranuclear gaze palsy, epilepsy, extrapyramidal features, progressive dementia, psychiatric manifestations	(Desnick 2004, Lachmann et al. 2004, Beck 2007)
Pompe	α-glucosidase	Glycogen	No	Progressive cardiomyopathy, skeletal muscle weakness, respiratory insufficiency	(Desnick 2004, Beck 2007)
Sandhoff	β-hexosaminidase B	Gangliosides (GM2)	Yes	Motor weakness, blindness, decerebrate rigidity	(Desnick 2004, Cachon-Gonzalez et al. 2006, Beck 2007)
Tay Sachs	β-hexosaminidase A	Gangliosides (GM2)	Yes	Motor weakness, blindness, decerebrate rigidity	(Desnick, 2004, Cachon-Gonzalez et al. 2006, Beck 2007)
Wolman	Acid lipase	Cholesterol esters and triglycerides	May affect myelination	Progressive feeding intolerance, diarrhea, massive hepatosplenomegaly, liver cirrhosis, pulmonary infiltrates and enlarged adrenal glands with punctate calcification	(Desnick 2004, Beck 2007, Stein et al. 2007)

β-galactosidase and β-N-acetylglucosaminidase to expose appropriate monosaccharide residues on the enzyme led to its increased uptake by macrophages mediated by the mannose receptor (Furbish et al. 1981). After initial studies showed low levels of therapeutic affects, the dosage was increased until liver and spleen enlargement were reversed (Barton et al. 1990, 1991). However, for ERT to be used broadly, large amounts of enzyme would be needed. Purification of β-glucosidase from human placenta was the original source, but as use of this therapy increased, recombinant enzyme generated using a Chinese hamster ovary cell line proved effective (Grabowski et al. 1995, Desnick 2004).

With similar ERT approaches, clinical trials demonstrated promise for other LSDs, including therapeutic intervention in Fabry disease (Schiffmann et al. 2000, 2001, Desnick 2004) and Mucopolysaccharidosis I (MPS I) (Kakkis et al. 2001, Wraith et al. 2004). Currently, a database provided by the U.S. National Library of Medicine (http://www.clinicaltrials.gov), lists six ERT studies in Phase III and IV for Fabry disease and two more for the Mucopolysachcharidoses. Thus, the successes obtained using ERT show it to be a valid therapeutic option for a number of LSDs. Specifically, ERT appears well tolerated and effective in visceral manifestations of LSDs due to ease of enzyme delivery *via* the bloodstream (Desnick 2004, Beck 2007). However, enzyme availability and trafficking (delivery) continue to be hurdles for widespread use of ERT (Desnick 2004).

What do these studies suggest regarding ERT for central nervous system (CNS) pathologies, including those exhibited by NCLs? Attempts to use ERT for CNS diseases show some promise. For instance, human brain myelination is increased in response to ERT for Pompe disease (Chien et al. 2006). Animal models have shown improvements in neuropathy associated with MPS VII following ERT (Vogler et al. 1999). Also, marked improvement in substrate clearing in the brain of a mouse model of α-Mannosidosis was achieved following intravenous delivery of the bovine isoform of α-mannosidase (Roces et al. 2004). In a related study using a guinea-pig model of α-Mannosidosis, preparations of recombinant human α-mannosidase purified with a new monoclonal antibody increased the half-life of the enzyme in the bloodstream after intravenous injection. As a result of the higher effective titer, the enzyme was able to cross the BBB (Crawley et al. 2006). Although there was a decrease in lysosomal storage material within the CNS, histological improvements in brain tissue were not observed (Crawley et al. 2006).

Pathology of the nervous system due to other LSDs (Table II) has been addressed with ERT; however, the BBB renders ERT less effective for diseases with CNS involvement. Therefore, few studies have employed traditional ERT for the treatment of NCLs (Hobert and Dawson 2006). One study validates the uptake of human enzyme by primary cultured rat cerebellar granule neurons (Lin and Lobel 2001). This work establishes the feasibility of effective ERT, but does not address methods of enzyme delivery. Attenuation of neurological symptoms in a $CLN2^{-}$- mouse model has recently been reported when investigators used intraventricular delivery of tripeptidyl peptidase I (TPP1); mutated TPP1 is responsible for the Late Infantile form of NCL (LINCL) (Chang et al. 2008). Intrathecal delivery of ERT does show promise in a MPS I canine model (Kakkis et al. 2004, Dickson et al. 2007). Also, the use of convection-enhanced distribution of enzymes during delivery (Bobo et al. 1994) has shown appropriate delivery of enzyme in rodent and primate models of Gaucher Disease (Lonser et al. 2007). Recent studies suggest ERT may be most effective for treating neuropathologies when combined with enzyme enhancement, gene therapy, cell-based transplants and/or pharmacological agents that permeate the blood brain barrier (Desnick 2004, Beck 2007).

GENE THERAPY

Gene therapy can refer to an *ex vivo* genetic modification of donor cells or to the introduction of a gene into host cells *in vivo*. For example, white blood cells can be removed from a patient, transduced with a viral vector containing a 'corrected' gene and reintroduced to the patient. The first clinical trial using gene therapy involved a child with severe combined immunodeficiency syndrome (SCID) and used a viral delivery system to transduce *ex vivo* lymphocytes with the normal gene for adenosine deaminase, an enzyme deficient in SCID patients responsible for the breakdown of purines. The transduced cells were transplanted back into the patient with therapeutic success (Thompson 1993). A drawback of this first trial was the necessity of repeated transplants due to short survival of the modified lymphocytes.

When gene therapy involves the direct 'infection' of cells *in vivo* by a viral vector, the virus is typically altered to reduce its virulence to avoid pathogenicity.

Below, we examine precedents of gene therapy that employed viral vectors to bring modified genes/enzymes into the CNS.

Chung and colleagues (Chung et al. 2007) used a murine model of MPS I to test two systemic levels of transgene expression with a retroviral vector encoding α-L-iduronidase. In its mutant form, this enzyme causes MPS I in humans. When the vector was delivered intravenously at high doses, a complete correction of the lysosomal storage defect was observed in peripheral tissues as well as a nearly complete correction in brain structures. At lower doses, only partial visceral correction was obtained and little therapeutic effect was found in the brain. In agreement with the results of Crawley and others (2006), described above, efficacy in the brain at high doses were likely dependent on increased enzyme diffusion across the BBB (Chung et al. 2007).

When gene therapy was applied directly to brain tissue of mice with Sandhoff Disease, a close relative of Tay-Sachs, it showed therapeutic benefit (Cachon-Gonzalez et al. 2006). In this study, an adeno-associated viral vector was used to deliver β-hexosaminidase subunit genes α and β. A single striatal injection of the vector induced expression of the subunits and dramatically reduced glycosphingolipid storage. This was associated with increased survival and improved neurological function. The survival of treated mice beyond one year marked a significant improvement compared to previous work on Sandhoff Disease using substrate inhibitors and bone marrow transplant (Jeyakumar et al. 2001). A clinical trial of substrate inhibitors for Tay Sachs patients did not demonstrate similar success (Bembi et al. 2006), but this may be attributable to either the increased complexity of the human brain, when compared to model systems or timing of therapeutic delivery relative to disease onset.

In studies of gene therapy applied to LSDs, a recombinant adenovirus encoding the gene for Niemann-Pick 1 (NPC I) protein demonstrated efficacy when applied *in vitro* to primary neural cultures (Paul et al. 2005). Subsequent experiments showed appropriate expression of NPC I upon injection of recombinant adenovirus into murine cerebellum, but the functional efficacy of this treatment was not addressed (Paul et al. 2005). A more recent study by Passini and colleagues (2007) using a mouse model of Niemann-Pick and recombinant adeno-associated viral vectors encoding human ASM, demonstrated global reversal of pathology when CNS injections were made at multiple sites and combined with systemic injections.

Sands and colleagues (Griffey et al. 2004) applied gene therapy directly to brains of a mouse knockout model for Infantile Neuronal Ceroid-Lipofuscinosis (INCL) (Table I). INCL is caused by mutations in palmitoyl protein thioesterase-1 (PPT1). Adeno-associated virus 2 (AAV2) delivery of human PPT1 was tested after intracranial injection. Seven months after the injections, they observed a significant decrease in lysosomal storage material and a localized slowing of the pathological phenotype (Griffey et al. 2004). A similar study by Passini and coauthors (2006) used AAV2 and AAV5 to direct production of tripeptidyl peptidase I TPP1 into CLN2^{-}-mice. At 13 weeks post-injection, a significant reduction in lysosomal storage material was observed when compared to age-matched control animals (Passini et al. 2006). It appears that AAV2 transduction of TPP1 in the striatum employs neural projections to transfer enzyme production to many other areas of the brain in rat and primate models of LINCL (Sondhi et al. 2005). Passini and colleagues (2006) using a mouse LINCL model demonstrated the necessity of early intervention (before symptom onset) for gene therapy to preserve motor neuron function. In a related study, this group demonstrated a reduction of storage material, but significant recovery did not occur if therapy was applied after onset of symptoms (Cabrera-Salazar et al. 2007). Following successes in these animal models, the safety of gene vectors for therapeutic applications to treat human LINCL is being addressed in a Phase I clinical trial by Crystal and colleagues (Crystal et al. 2007).

The progress in gene therapy using viral vectors shows great promise for the treatment of LSDs. Viral delivery allows prolonged protein production by cells of an affected tissue (long after the therapeutic value of ERT alone would have declined). Also, by using host cells for endogenous protein production, appropriate post-translational modifications and regulation may further increase efficacy. In addition as noted above, viral transmission provides long-term production of enzyme in the recipient, eliminating the need for repeated enzyme treatments (Chung et al. 2007). The success obtained in animal models provides the foundation for translational studies in clinical trials. However, the increased size of the human brain along with immunological rejection of vectors and vector products are untested and may present significant hurdles for future

therapies (Hobert and Dawson 2006). Additional work must also be done to address the long-term expression of transgene products. This may be an issue of virus or vector selection (Yee and Zaia 2001, Jenke et al. 2004). Natural viral pseudotypes or molecular manipulations of a vector can result in broad variations of each vector with regard to immunotoxicity, transfer capacity, stability *in vivo* and efficacy (Osten et al. 2007).

CELL-MEDIATED THERAPY

Cell transplant therapy can include a number of approaches, involving different types of donor cells (some genetically modified) and delivery methods. Donor cells, such as stem cells, can be transplanted with the purpose of cellular replacement after loss due to disease or injury or can be used as a source of cellular product lacking in the host. In addition, donor cells can provide trophic factors that promote survival of defective host cells. Cellular therapies have been tested in several disease models. Here we discuss select examples that highlight the promise and challenges of this approach to treating LSDs, including the NCLs.

Bone marrow transplants (BMT) or transplants of bone marrow-derived stem cells (BMSCs) provide a unique source of cells for cell-mediated therapies. Some studies suggest that a limited number of BMSCs can cross the blood brain barrier, integrate in the brain and differentiate into neural cells after BMTs (Tanaka et al. 2003, Bae et al. 2007, Sostak et al. 2007). There is debate as to whether this represents a true differentiation of the BMSCs into neural cells, especially since some of these results are due to fusion of BMSCs with host neurons (Bae et al. 2005, Pierret et al. 2006). Nevertheless, successful engraftment after BMT can produce therapeutic affects in the CNS (Boelens 2006). For instance, donor cell engraftment after BMT in Hurler patients leads to effects in both visceral and CNS systems including improved mental development and relief from airway obstruction (Boelens 2006).

Umbilical cord blood (UCB) presents another source for cell-mediated therapies. Immunological privilege allows some cases of HLA-mismatched UCB transplants to provide long-term engraftment of donated cells. Use of UCB transplant into at least one patient with Wolman Disease has led to a significant therapeutic response for more than four years (Stein et al. 2007). Moreover, UCB cells may improve the outcome of many LSDs if response is early (Boelens 2006).

Each of the previous examples highlights the use of donor cells to engraft and produce physiological levels of an enzyme or trophic factors to enhance the survival of host cells. Two more options to enhance the therapeutic benefits of cell-mediated therapy include: (1) the use of common transfection or transduction techniques that modify allogeneic donor cells to regulate production of an enzyme. For instance, transgenic over expression will likely increase production of a soluble enzyme product that may result in enhanced product distribution; and (2) transgenic modification of the recipient's cells *ex vivo* prior to transplantation. Subsequent engraftment of transgenic cells can lead to cross-correction of the cellular pathology. In one study, retroviral transduction of *ex vivo* syngeneic bone marrow cells followed by BMT into type 1 Gaucher mice produced enzyme levels similar to those demonstrated by wild-type BMT and resulted in significant cross-correction of the disease (Enquist et al. 2006). Thus, syngeneic or autologous transplantation may become a realistic approach for cell-mediated treatment of a number of LSDs.

Over expression of enzyme by transgenic neural stem cells has been effective in cell-mediated therapies for LSDs. In the case of MPS VII, immortalized human NSCs engineered to produce β-glucuronidase were transplanted into the MPS VII model mouse brain and resulted in a reduction of glycosaminoglycan substrate and significant correction of diffuse CNS lesions (Meng et al. 2003). In another application of cell-mediated therapy, transgenic over expression of arylsulfatase A by microglial progeny of hematopoietic stem cells was effective in correction of metachromatic leukodystrophy that was correlated with reduction of lysosomal storage material (Biffi et al. 2004). In a related study, peripheral over expression of arylsulfatase A by transgenic hepatocytes alone was ineffective in improving CNS pathology, indicating that cell migration across the blood brain barrier is necessary (Biffi et al. 2006).

An interesting example of an alternative cell-mediated approach is to encapsulate immortalized human amniotic epithelial cells that produce β-glucoronidase when transplanted into the MPS VII mouse brain (Nakama et al. 2006). With this method, the encapsulated cells do not have physical contact with host cells and are rendered incapable of unchecked proliferation. This approach demonstrates a therapeutic benefit and circumvents the problem of CNS access as well as the potential risk of teratoma formation if undifferentiated embryonic stem cells are used. It may be important to

consider dosage and employ a transgenic control for the secreted product. For instance, doxycycline regulated vectors may address this issue, even in the CNS (Stieger et al. 2007).

There is one published case in which BMT was employed for two patients with NCLs, one with the late infantile and one with the juvenile onset of the disease (Lake et al. 1997). Therapeutic value of the transplant was not apparent at the time of the publication and has not since been updated. Efficacy of cell transplants and other methods may be obscured in early clinical trials as patients are often selected with advanced stages of the disease. Bone marrow transplantation is in Phase II trial with regard to NCLs and LSDs at large (Orchard 2007).

The successes of cell-mediated therapies in models of other LSDs led to a human clinical trial currently underway in which human neural stem cells have been transplanted into the brains of children with Batten's disease (Hobert and Dawson 2006, Taupin 2006). This type of therapeutic approach enlists the ability of transplanted neural progenitors to incorporate and produce factors lacking in host cells, thus sparing the latter from excessive lysosomal storage and cell death.

In a recent study, we observed integration of donor stem cells into the eyes of a neurodegenerative model for the NCLs (Meyer et al. 2006). After neural induction mouse embryonic stem cells were transplanted into the vitreous of the *mnd* mouse. The *mnd* mouse is a spontaneous mutant with homozygous recessive inheritance of the CLN8 gene (Bronson et al. 1993, Ranta et al. 1999). Successful integration and differentiation of donor stem cells into the neural retina was observed, and donor cells exhibited the morphologies and expressed markers expected of retinal neurons, including horizontal cells, ganglion cells, bipolar cells, and amacrine cells but no definitive photoreceptors. A therapeutic benefit provided by the donor cells was observed with enhanced survival of host photoreceptors associated with a significant reduction in number and volume of lysosomal storage bodies. Current work on this NCL model is focused on increased production of neurotrophic factors by donor stem cells.

Cell-mediated therapies for LSDs bridge the applications of ERT and gene therapy. If replacement of specific cell-types is required to treat an advanced disease or injury condition, the source of cells, their migration to sites of degeneration and ability to differentiate into appropriate cell types should be considered.

PHARMACOLOGICAL/SMALL MOLECULE INTERVENTION

Pharmacological intervention has been successful for some LSDs, although, depending on the particular treatment, effectiveness may be limited to one or few disorders. Cysteamine prevents or significantly delays cystinosis (Kleta and Gahl 2004), but is not appropriate for other LSDs. Cyclosporin A, however, inhibits the translocation of glucosyl ceramide in Gaucher cell lines and *de novo* synthesis of globotriaosyl ceramide in Fabry cell lines, and when delivered to mouse models results in reduced accumulation of storage material and considerable recovery from both disease phenotypes (Mattocks et al. 2006). Because of its broader success, this approach may be considered as an adjunct to other treatments for glycosphingolipid storage diseases.

Previously, the small molecule miglustat (N-butyldeoxynojirimycin), an inhibitor of GSL biosynthesis, was administered to a patient with Niemann-Pick Type C, possibly arresting the advancement of symptoms, though this conclusion must certainly be documented in additional studies (Lachmann et al. 2004). More recently, various therapeutic benefits, including CNS and visceral improvement were reported following the administration of Miglustat to two children with Niemann-Pick Type C (Chien et al. 2007). Miglustat also extends survival of Niemann-Pick Type C model mice (Zervas et al. 2001). These findings were significant, as miglustat has no direct effect on cholesterol metabolism itself and therefore was determined useful for treating other LSDs. It is now a licensed product for the treatment of LSDs (FDA 2006).

Pharmacological and small molecule therapeutic interventions may act as agonists or antagonists to specific ligands or receptors in the lysosome, but errors in folding or trafficking of an enzyme in the lysosome may also lead to LSD. In the latter case, designing molecular chaperones may improve the residual function of mutant proteins (Desnick 2004). However, this approach may be difficult, as a unique chaperone may be necessary for each disorder.

Treatment of NCLs has also been approached with pharmacological agents. The *mnd* mouse shows enhanced survival of motor neurons and maintenance of motor function when treated with Clenbuteral (a β_2-adrenoceptor agonist). Clenbuteral also enhances axon regeneration of lesioned motor neurons. However, the mechanism(s) whereby Clenbuteral achieves these benefits is not

known (Zeman et al. 2004). More recent work supports the S^+-enantiomer but not R^--clenbuterol neuroprotective effects on cultured cells (Culmsee et al. 2007). Zhang and coworkers (2001) have shown that use of phosphocysteamine, that preferentially targets the lysosome, can prevent the re-accumulation of storage materials and delay cell death in lymphoblasts derived from INCL patients, likely due to depletion of lysosomal ceroid material. Cysteamine (Cystagon) has gained attention in the treatment of INCL by reducing substrate accumulation. However, little benefit on the accumulation of lysosomal storage occurs unless such lysosomotrophic drugs are used in combination with another therapeutic agent (Lu et al. 2002, Hobert and Dawson 2006). Most recently, Cystagon has been combined with N-Acetylcysteine (Mucomyst) for the treatment of children with INCL. This trial has entered Phase II (NICHD 2007).

Pharmaceutical and small molecule treatments for LSDs and NCLs appear likely to be successful in some cases and thus deserve consideration when developing new therapies for these disorders. They may act upstream of lysosomal storage to reduce a substrate or later in the pathway to enhance enzyme activity by functioning as molecular chaperones (Beck 2007). High specificity of small molecule interactions, however, will require an in depth knowledge of the pathology and etiology of each disorder.

CONCLUSIONS

Effective therapeutic options are on the horizon for LSDs, each with its own benefits and limitations. ERT shows promise for treatment of visceral pathologies in these diseases when replacement of soluble enzyme is sufficient for a therapeutic response. Optimization of enzyme titer to produce the best patient response will continue to be a major focus of ERT for CNS presentation of LSDs. The success of ERT on type 1 Gaucher disease (Brady et al. 1974, Desnick 2004) provides a renewed excitement for application of ERTs to treat LSDs, including certain NCLs.

A major advantage of gene therapy *via* viral delivery is prolonged production of therapeutic agents, especially when compared to intravenous delivery of an enzyme. Gene therapy is limited by its inability to act over large areas of the brain (Hobert and Dawson 2006), and there are also concerns related to potential recombination events that could result in undesirable cellular transformation (Glover et al. 2005).

Ex vivo gene therapy, regardless of vector type, has proven effective in transduction of various cell types for production of recombinant enzyme (Meng et al. 2003, Meyer et al. 2006). Cell-mediated therapies can combine ERT and gene therapy, but must overcome issues such as potential tumorogenecity, appropriate integration of donor cells within the host and ability of transplanted cells to function physiologically in a manner equivalent to endogenous cells.

Because pharmaco-therapy does not typically address the causative mutation of a disorder it may best be employed as an adjunct to these approaches. It could act to reduce the negative impact of pathological cellular events associated with abnormal accumulation of products within lysosomes.

With respect to current therapeutic approaches to treat NCLs, we suggest that considering applications for LSDs as a whole may derive novel therapies. For instance, disorders with CNS involvement will benefit from a broader spectrum of approaches that address the BBB as well as the size of the human brain as compared to that of rodent NCL models. The combination of transgenic cell-mediated approaches that provide long-term engraftment and therapeutic delivery of an enzyme and selective pharmacological agents that reduce the abnormal buildup of lysosomal storage bodies may provide the best therapeutic outcome.

ACKNOWLEDGEMENTS

We thank Dr. Martin Katz for his contributions to the section on Pharmacological and Small Molecule Intervention. This work was supported by NIH (MDK) and a MU Graduate Life Sciences Fellowship (CP).

REFERENCES

Bae JS, Furuya S, Shinoda Y, Endo S, Schuchman EH, Hirabayashi Y, Jin HK (2005) Neurodegeneration augments the ability of bone marrow-derived mesenchymal stem cells to fuse with Purkinje neurons in Niemann-Pick type C mice. Hum Gene Ther 16: 1006–1011.

Bae JS, Han HS, Youn DH, Carter JE, Modo M, Schuchman EH, Jin HK (2007) Bone marrow-derived mesenchymal stem cells promote neuronal networks with functional synaptic transmission after transplantation into mice with neurodegeneration. Stem Cells 25: 1307–1316.

Barton NW, Furbish FS, Murray GJ, Garfield M, Brady RO (1990) Therapeutic response to intravenous infusions of glucocerebrosidase in a patient with Gaucher disease. Proc Natl Acad Sci U S A 87: 1913–1916.

Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, et al. (1991) Replacement therapy for inherited enzyme deficiency--macrophage-targeted glucocerebrosidase for Gaucher's disease. N Engl J Med 324: 1464–1470.

Beck M (2007) New therapeutic options for lysosomal storage disorders: enzyme replacement, small molecules and gene therapy. Hum Genet 121: 1–22.

Bembi B, Marchetti F, Guerci VI, Ciana G, Addobbati R, Grasso D, Barone R, Cariati R, Fernandez-Guillen L, Butters T, Pittis MG (2006) Substrate reduction therapy in the infantile form of Tay-Sachs disease. Neurology 66: 278–280.

Biffi A, De Palma M, Quattrini A, Del Carro U, Amadio S, Visigalli I, Sessa M, Fasano S, Brambilla R, Marchesini S, Bordignon C, Naldini L (2004) Correction of metachromatic leukodystrophy in the mouse model by transplantation of genetically modified hematopoietic stem cells. J Clin Invest 113: 1118–1129.

Biffi A, Capotondo A, Fasano S, del Carro U, Marchesini S, Azuma H, Malaguti MC, Amadio S, Brambilla R, Grompe M, Bordignon C, Quattrini A, Naldini L (2006) Gene therapy of metachromatic leukodystrophy reverses neurological damage and deficits in mice. J Clin Invest 116: 3070–3082.

Bobo RH, Laske DW, Akbasak A, Morrison PF, Dedrick RL, Oldfield EH (1994) Convection-enhanced delivery of macromolecules in the brain. Proc Natl Acad Sci U S A 91: 2076–2080.

Boelens JJ (2006) Trends in haematopoietic cell transplantation for inborn errors of metabolism. J Inherit Metab Dis 29: 413–420.

Brady RO, Pentchev PG, Gal AE, Hibbert SR, Dekaban AS (1974) Replacement therapy for inherited enzyme deficiency. Use of purified glucocerebrosidase in Gaucher's disease. N Engl J Med 291: 989–993.

Bronson RT, Lake BD, Cook S, Taylor S, Davisson MT (1993) Motor neuron degeneration of mice is a model of neuronal ceroid lipofuscinosis (Batten's disease). Ann Neurol 33: 381–385.

Cabrera-Salazar MA, Roskelley EM, Bu J, Hodges BL, Yew N, Dodge JC, Shihabuddin LS, Sohar I, Sleat DE, Scheule RK, Davidson BL, Cheng SH, Lobel P, Passini MA (2007) Timing of therapeutic intervention determines functional and survival outcomes in a mouse model of late infantile batten disease. Mol Ther 15: 1782–1788.

Cachon-Gonzalez MB, Wang SZ, Lynch A, Ziegler R, Cheng SH, Cox TM (2006) Effective gene therapy in an authentic model of Tay-Sachs-related diseases. Proc Natl Acad Sci U S A 103: 10373–10378.

Chang M, Cooper JD, Sleat DE, Cheng SH, Dodge JC, Passini MA, Lobel P, Davidson BL (2008) Intraventricular enzyme replacement improves disease phenotypes in a mouse model of late infantile neuronal ceroid lipofuscinosis. Mol Ther 16: 649–656.

Chien YH, Lee NC, Peng SF, Hwu WL (2006) Brain development in infantile-onset Pompe disease treated by enzyme replacement therapy. Pediatr Res 60: 349–352.

Chien YH, Lee NC, Tsai LK, Huang AC, Peng SF, Chen SJ, Hwu WL (2007) Treatment of Niemann-Pick disease type C in two children with miglustat: initial responses and maintenance of effects over 1 year. J Inherit Metab Dis 30: 826.

Chung S, Ma X, Liu Y, Lee D, Tittiger M, Ponder KP (2007) Effect of neonatal administration of a retroviral vector expressing alpha-L-iduronidase upon lysosomal storage in brain and other organs in mucopolysaccharidosis I mice. Mol Genet Metab 90: 181–192.

Cooper JD, Russell C, Mitchison HM (2006) Progress towards understanding disease mechanisms in small vertebrate models of neuronal ceroid lipofuscinosis. Biochim Biophys Acta 1762: 873–889.

Crawley AC, King B, Berg T, Meikle PJ, Hopwood JJ (2006) Enzyme replacement therapy in alpha-mannosidosis guinea-pigs. Mol Genet Metab 89: 48–57.

Crystal R, Weill Medical College of Cornell University, Foundation NsB (2007) Safety Study of a Gene Transfer Vector for Children With Late Infantile Neuronal Ceroid Lipofuscinosis. At http://clinicaltrials.gov/show/NCT00151216, National Library of Medicine (US), Accessed 25 February, 2008.

Culmsee C, Junker V, Thal S, Kremers W, Maier S, Schneider HJ, Plesnila N, Krieglstein J (2007) Enantio-selective effects of clenbuterol in cultured neurons and astrocytes, and in a mouse model of cerebral ischemia. Eur J Pharmacol 575: 57–65.

Desnick RJ, Dean KJ, Grabowski GA, Bishop DF, Sweeley CC (1980) Enzyme therapy XVII: metabolic and immunologic evaluation of alpha- galactosidase A replacement in Fabry disease. Birth Defects Orig Artic Ser 16: 393–413.

Desnick RJ (2004) Enzyme replacement and enhancement therapies for lysosomal diseases. J Inherit Metab Dis 27: 385–410.

Dickson P, McEntee M, Vogler C, Le S, Levy B, Peinovich M, Hanson S, Passage M, Kakkis E (2007) Intrathecal enzyme replacement therapy: successful treatment of brain disease via the cerebrospinal fluid. Mol Genet Metab 91: 61–68.

Enquist IB, Nilsson E, Ooka A, Mansson JE, Olsson K, Ehinger M, Brady RO, Richter J, Karlsson S (2006) Effective cell and gene therapy in a murine model of Gaucher disease. Proc Natl Acad Sci U S A 103: 13819–13824.

(FDA) U.S. Food and Drug Administration (2006) First treatment for Pompe disease. FDA Consum 40: 3.

Furbish FS, Steer CJ, Krett NL, Barranger JA (1981) Uptake and distribution of placental glucocerebrosidase in rat hepatic cells and effects of sequential deglycosylation. Biochim Biophys Acta 673: 425–434.

Futerman AH, van Meer G (2004) The cell biology of lysosomal storage disorders. Nat Rev Mol Cell Biol 5: 554–565.

Gao H, Boustany RM, Espinola JA, Cotman SL, Srinidhi L, Antonellis KA, Gillis T, Qin X, Liu S, Donahue LR, Bronson RT, Faust JR, Stout D, Haines JL, Lerner TJ, MacDonald ME (2002) Mutations in a novel CLN6-encoded transmembrane protein cause variant neuronal ceroid lipofuscinosis in man and mouse. Am J Hum Genet 70: 324–335.

Glover DJ, Lipps HJ, Jans DA (2005) Towards safe, non-viral therapeutic gene expression in humans. Nat Rev Genet 6: 299–310.

Grabowski GA, Barton NW, Pastores G, Dambrosia JM, Banerjee TK, McKee MA, Parker C, Schiffmann R, Hill SC, Brady RO (1995) Enzyme therapy in type 1 Gaucher disease: comparative efficacy of mannose-terminated glucocerebrosidase from natural and recombinant sources. Ann Intern Med 122: 33–39.

Griffey M, Bible E, Vogler C, Levy B, Gupta P, Cooper J, Sands MS (2004) Adeno-associated virus 2-mediated gene therapy decreases autofluorescent storage material and increases brain mass in a murine model of infantile neuronal ceroid lipofuscinosis. Neurobiol Dis 16: 360–369.

Haltia M (2006) The neuronal ceroid-lipofuscinoses: from past to present. Biochim Biophys Acta 1762: 850–856.

Hobert JA, Dawson G (2006) Neuronal ceroid lipofuscinoses therapeutic strategies: past, present and future. Biochim Biophys Acta 1762: 945–953.

(IBDC) The International Batten Disease Consortium (1995) Isolation of a novel gene underlying Batten disease, CLN3. Cell 82: 949–957.

Jalanko A, Tyynela J, Peltonen L (2006) From genes to systems: new global strategies for the characterization of NCL biology. Biochim Biophys Acta 1762: 934–944.

Jenke AC, Scinteie MF, Stehle IM, Lipps HJ (2004) Expression of a transgene encoded on a non-viral episomal vector is not subject to epigenetic silencing by cytosine methylation. Mol Biol Rep 31: 85–90.

Jeyakumar M, Norflus F, Tifft CJ, Cortina-Borja M, Butters TD, Proia RL, Perry VH, Dwek RA, Platt FM (2001) Enhanced survival in Sandhoff disease mice receiving a combination of substrate deprivation therapy and bone marrow transplantation. Blood 97: 327–329.

Johnson WG, Desnick RJ, Long DM, Sharp HL, Krivit W, Brady B, Brady RO (1973) Intravenous injection of purified hexosaminidase A into a patient with Tay-Sachs disease. Birth Defects Orig Artic Ser 9: 120–124.

Kacher Y, Futerman AH (2006) Genetic diseases of sphingolipid metabolism: pathological mechanisms and therapeutic options. FEBS Lett 580: 5510–5517.

Kakkis ED, Muenzer J, Tiller GE, Waber L, Belmont J, Passage M, Izykowski B, Phillips J, Doroshow R, Walot I, Hoft R, Neufeld EF (2001) Enzyme-replacement therapy in mucopolysaccharidosis I. N Engl J Med 344: 182–188.

Kakkis E, McEntee M, Vogler C, Le S, Levy B, Belichenko P, Mobley W, Dickson P, Hanson S, Passage M (2004) Intrathecal enzyme replacement therapy reduces lysosomal storage in the brain and meninges of the canine model of MPS I. Mol Genet Metab 83: 163–174.

Kleta R, Gahl WA (2004) Pharmacological treatment of nephropathic cystinosis with cysteamine. Expert Opin Pharmacother 5: 2255–2262.

Kornfeld S, Mellman I (1989) The biogenesis of lysosomes. Annu Rev Cell Biol 5: 483–525.

Kyttala A, Lahtinen U, Braulke T, Hofmann SL (2006) Functional biology of the neuronal ceroid lipofuscinoses (NCL) proteins. Biochim Biophys Acta 1762: 920–933.

Lachmann RH, te Vruchte D, Lloyd-Evans E, Reinkensmeier G, Sillence DJ, Fernandez-Guillen L, Dwek RA, Butters TD, Cox TM, Platt FM (2004) Treatment with miglustat reverses the lipid-trafficking defect in Niemann-Pick disease type C. Neurobiol Dis 16: 654–658.

Lake BD, Steward CG, Oakhill A, Wilson J, Perham TG (1997) Bone marrow transplantation in late infantile Batten disease and juvenile Batten disease. Neuropediatrics 28: 80–81.

Lin L, Lobel P (2001) Production and characterization of recombinant human CLN2 protein for enzyme-replacement therapy in late infantile neuronal ceroid lipofuscinosis. Biochem J 357: 49–55.

Lonser RR, Schiffman R, Robison RA, Butman JA, Quezado Z, Walker ML, Morrison PF, Walbridge S, Murray GJ, Park DM, Brady RO, Oldfield EH (2007) Image-guided, direct

convective delivery of glucocerebrosidase for neuronopathic Gaucher disease. Neurology 68: 254–261.

Lu JY, Verkruyse LA, Hofmann SL (2002) The effects of lysosomotropic agents on normal and INCL cells provide further evidence for the lysosomal nature of palmitoyl-protein thioesterase function. Biochim Biophys Acta 1583: 35–44.

Mattocks M, Bagovich M, De Rosa M, Bond S, Binnington B, Rasaiah VI, Medin J, Lingwood C (2006) Treatment of neutral glycosphingolipid lysosomal storage diseases via inhibition of the ABC drug transporter, MDR1. Cyclosporin A can lower serum and liver globotriaosyl ceramide levels in the Fabry mouse model. Febs J 273: 2064–2075.

Meng XL, Shen JS, Ohashi T, Maeda H, Kim SU, Eto Y (2003) Brain transplantation of genetically engineered human neural stem cells globally corrects brain lesions in the mucopolysaccharidosis type VII mouse. J Neurosci Res 74: 266–277.

Meyer JS, Katz ML, Maruniak JA, Kirk MD (2006) Embryonic stem cell-derived neural progenitors incorporate into degenerating retina and enhance survival of host photoreceptors. Stem Cells 24: 274–283.

Nakama H, Ohsugi K, Otsuki T, Date I, Kosuga M, Okuyama T, Sakuragawa N (2006) Encapsulation cell therapy for mucopolysaccharidosis type VII using genetically engineered immortalized human amniotic epithelial cells. Tohoku J Exp Med 209: 23–32.

(NICHD) National Institute of Child Health and Human Development (2007) Cystagon to Treat Infantile Neuronal Ceroid Lipofuscinosis. In http://clinicaltrials.gov/show/NCT00028262, National Library of Medicine (US), Accessed 25February, 2008.

Orchard P (2007) Stem Cell Transplant for Inborn Errors of Metabolism. At http://clinicaltrials.gov/show/NCT00176904, National Library of Medicine (US), Accessed 25 February, 2008.

Osten P, Grinevich V, Cetin A (2007) Viral vectors: a wide range of choices and high levels of service. Handb Exp Pharmacol: 177–202.

Passini MA, Dodge JC, Bu J, Yang W, Zhao Q, Sondhi D, Hackett NR, Kaminsky SM, Mao Q, Shihabuddin LS, Cheng SH, Sleat DE, Stewart GR, Davidson BL, Lobel P, Crystal RG (2006) Intracranial delivery of CLN2 reduces brain pathology in a mouse model of classical late infantile neuronal ceroid lipofuscinosis. J Neurosci 26: 1334–1342.

Passini MA, Bu J, Fidler JA, Ziegler RJ, Foley JW, Dodge JC, Yang WW, Clarke J, Taksir TV, Griffiths DA, Zhao MA, O'Riordan CR, Schuchman EH, Shihabuddin LS, Cheng SH (2007) Combination brain and systemic injections of AAV provide maximal functional and survival benefits in the Niemann-Pick mouse. Proc Nat Acad Sci (USA) 104: 9505–9510.

Paul CA, Reid PC, Boegle AK, Karten B, Zhang M, Jiang ZG, Franz D, Lin L, Chang TY, Vance JE, Blanchette-Mackie J, Maue RA (2005) Adenovirus expressing an NPC1-GFP fusion gene corrects neuronal and nonneuronal defects associated with Niemann pick type C disease. J Neurosci Res 81: 706–719.

Pierret C, Spears K, Maruniak JA, Kirk MD (2006) Neural crest as the source of adult stem cells. Stem Cells Dev 15: 286–291.

Piotrowska E, Jakobkiewicz-Banecka J, Baranska S, Tylki-Szymanska A, Czartoryska B, Wegrzyn A, Wegrzyn G (2006) Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. Eur J Hum Genet 14: 846–852.

Ranta S, Zhang Y, Ross B, Lonka L, Takkunen E, Messer A, Sharp J, Wheeler R, Kusumi K, Mole S, Liu W, Soares MB, Bonaldo MF, Hirvasniemi A, de la Chapelle A, Gilliam TC, Lehesjoki AE (1999) The neuronal ceroid lipofuscinoses in human EPMR and *mnd* mutant mice are associated with mutations in CLN8. Nat Genet 23: 233–236.

Reczek D, Schwake M, Schroder J, Hughes H, Blanz J, Jin X, Brondyk W, Van Patten S, Edmunds T, Saftig P (2007) LIMP-2 is a receptor for lysosomal mannose-6-phosphate-independent targeting of beta-glucocerebrosidase. Cell 131: 770–783.

Rider JA, Rider DL (1988) Batten disease: past, present, and future. Am J Med Genet Suppl 5: 21–26.

Roces DP, Lullmann-Rauch R, Peng J, Balducci C, Andersson C, Tollersrud O, Fogh J, Orlacchio A, Beccari T, Saftig P, von Figura K (2004) Efficacy of enzyme replacement therapy in alpha-mannosidosis mice: a preclinical animal study. Hum Mol Genet 13: 1979–1988.

Savukoski M, Klockars T, Holmberg V, Santavuori P, Lander ES, Peltonen L (1998) CLN5, a novel gene encoding a putative transmembrane protein mutated in Finnish variant late infantile neuronal ceroid lipofuscinosis. Nat Genet 19: 286–288.

Schiffmann R, Murray GJ, Treco D, Daniel P, Sellos-Moura M, Myers M, Quirk JM, Zirzow GC, Borowski M, Loveday K, Anderson T, Gillespie F, Oliver KL, Jeffries NO, Doo E, Liang TJ, Kreps C, Gunter K, Frei K, Crutchfield K, Selden RF, Brady RO (2000) Infusion of alpha-galactosidase A reduces tissue globotriaosylceramide storage in patients with Fabry disease. Proc Natl Acad Sci U S A 97: 365–370.

Schiffmann R, Kopp JB, Austin HA, 3rd, Sabnis S, Moore DF, Weibel T, Balow JE, Brady RO (2001) Enzyme replacement therapy in Fabry disease: a randomized controlled trial. JAMA 285: 2743–2749.

Schulz A, Dhar S, Rylova S, Dbaibo G, Alroy J, Hagel C, Artacho I, Kohlschutter A, Lin S, Boustany RM (2004) Impaired cell adhesion and apoptosis in a novel CLN9 Batten disease variant. Ann Neurol 56: 342–350.

Siintola E, Lehesjoki AE, Mole SE (2006a) Molecular genetics of the NCLs -- status and perspectives. Biochim Biophys Acta 1762: 857–864.

Siintola E, Partanen S, Stromme P, Haapanen A, Haltia M, Maehlen J, Lehesjoki AE, Tyynela J (2006b) Cathepsin D deficiency underlies congenital human neuronal ceroid-lipofuscinosis. Brain 129: 1438–1445.

Siintola E, Topcu M, Aula N, Lohi H, Minassian BA, Paterson AD, Liu XQ, Wilson C, Lahtinen U, Anttonen AK, Lehesjoki AE (2007) The novel neuronal ceroid lipofuscinosis gene MFSD8 encodes a putative lysosomal transporter. Am J Hum Genet 81: 136–146.

Sleat DE, Donnelly RJ, Lackland H, Liu CG, Sohar I, Pullarkat RK, Lobel P (1997) Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. Science 277: 1802–1805.

Sondhi D, Peterson DA, Giannaris EL, Sanders CT, Mendez BS, De B, Rostkowski AB, Blanchard B, Bjugstad K, Sladek JR, Jr., Redmond DE, Jr., Leopold PL, Kaminsky SM, Hackett NR, Crystal RG (2005) AAV2-mediated CLN2 gene transfer to rodent and non-human primate brain results in long-term TPP-I expression compatible with therapy for LINCL. Gene Ther 12: 1618–1632.

Sostak P, Theil D, Stepp H, Roeber S, Kretzschmar HA, Straube A (2007) Detection of bone marrow-derived cells expressing a neural phenotype in the human brain. J Neuropathol Exp Neurol 66: 110–116.

Stein J, Zion Garty B, Dror Y, Fenig E, Zeigler M, Yaniv I (2007) Successful treatment of Wolman disease by unrelated umbilical cord blood transplantation. Eur J Pediatr 166: 663–666.

Stieger K, Mendes-Madeira A, Meur GL, Weber M, Deschamps JY, Nivard D, Provost N, Moullier P, Rolling F (2007) Oral administration of doxycycline allows tight control of transgene expression: a key step towards gene therapy of retinal diseases. Gene Ther 14: 1668–1673.

Tanaka R, Komine-Kobayashi M, Mochizuki H, Yamada M, Furuya T, Migita M, Shimada T, Mizuno Y, Urabe T (2003) Migration of enhanced green fluorescent protein expressing bone marrow-derived microglia/macrophage into the mouse brain following permanent focal ischemia. Neuroscience 117: 531–539.

Taupin P (2006) HuCNS-SC (StemCells). Curr Opin Mol Ther 8: 156–163.

Thompson L (1993) The first kids with new genes. Time 141: 50–53.

van Meel E, Klumperman J (2008) Imaging and imagination: understanding the endo-lysosomal system. Histochem Cell Biol 129: 253–266.

Vesa J, Hellsten E, Verkruyse LA, Camp LA, Rapola J, Santavuori P, Hofmann SL, Peltonen L (1995) Mutations in the palmitoyl protein thioesterase gene causing infantile neuronal ceroid lipofuscinosis. Nature 376: 584–587.

Vogler C, Levy B, Galvin NJ, Thorpe C, Sands MS, Barker JE, Baty J, Birkenmeier EH, Sly WS (1999) Enzyme replacement in murine mucopolysaccharidosis type VII: neuronal and glial response to beta-glucuronidase requires early initiation of enzyme replacement therapy. Pediatr Res 45: 838–844.

Wheeler RB, Sharp JD, Schultz RA, Joslin JM, Williams RE, Mole SE (2002) The gene mutated in variant late-infantile neuronal ceroid lipofuscinosis (CLN6) and in nclf mutant mice encodes a novel predicted transmembrane protein. Am J Hum Genet 70: 537–542.

Wraith JE, Clarke LA, Beck M, Kolodny EH, Pastores GM, Muenzer J, Rapoport DM, Berger KI, Swiedler SJ, Kakkis ED, Braakman T, Chadbourne E, Walton-Bowen K, Cox GF (2004) Enzyme replacement therapy for mucopolysaccharidosis I: a randomized, double-blinded, placebo-controlled, multinational study of recombinant human alpha-L-iduronidase (laronidase). J Pediatr 144: 581–588.

Yee JK, Zaia JA (2001) Prospects for gene therapy using HIV-based vectors. Somat Cell Mol Genet 26: 159–174.

Zeman RJ, Peng H, Etlinger JD (2004) Clenbuterol retards loss of motor function in motor neuron degeneration mice. Exp Neurol 187: 460–467.

Zeman W, Dyken P (1969) Neuronal ceroid-lipofuscinosis (Batten's disease): relationship to amaurotic family idiocy? Pediatrics 44: 570–583.

Zervas M, Somers KL, Thrall MA, Walkley SU (2001) Critical role for glycosphingolipids in Niemann-Pick disease type C. Curr Biol 11: 1283–1287.

Zhang Z, Butler JD, Levin SW, Wisniewski KE, Brooks SS, Mukherjee AB (2001) Lysosomal ceroid depletion by drugs: therapeutic implications for a hereditary neurodegenerative disease of childhood. Nat Med 7: 478–484.