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-acetylgalactosamine-phosphate, then remove the N-acetylgalactosamine 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, and...
### Table I

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene Product</th>
<th>Product Type</th>
<th>Stored Protein</th>
<th>Onset</th>
<th>Clinical Presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLN1</td>
<td>PPT1 (palmitoyl protein thioesterase 1) (Vesa et al. 1995)</td>
<td>Soluble Enzyme</td>
<td>Sphingolipid activator proteins (SAPs)</td>
<td>Infantile, Late Infantile, Juvenile and Adult</td>
<td>Decreased head growth, retarded psychomotor development, epileptic seizures, muscular hypotonia, ataxia, visual failure, severe brain atrophy</td>
</tr>
<tr>
<td>CLN2</td>
<td>TPP1 (tripeptidyl peptidase 1) (Sleat et al. 1997)</td>
<td>Soluble Enzyme</td>
<td>Subunit c of Mitochondrial ATP synthase (SCMAS)</td>
<td>Late infantile, Juvenile and Protracted</td>
<td>Seizures, ataxia, myoclonus, developmental and mental retardation, visual failure, speech impairment</td>
</tr>
<tr>
<td>CLN3</td>
<td>CLN3/Battenin (IBDC 1995)</td>
<td>Lysosomal Transmembrane Protein</td>
<td>SCMAS</td>
<td>Juvenile, Adult</td>
<td>Progressive visual failure, blindness, seizures, psychomotor deterioration</td>
</tr>
<tr>
<td>CLN4</td>
<td>Unknown</td>
<td>Unknown</td>
<td>SCMAS</td>
<td>Adult</td>
<td>Dementia, progressive myoclonus epilepsy, ataxia, late pyramidal and extra-pyramidal features, behavioral changes, motor disturbances</td>
</tr>
<tr>
<td>CLN5</td>
<td>CLN5 (Savukoski et al. 1998)</td>
<td>Lysosomal Glycoprotein (may have soluble form)</td>
<td>SCMAS</td>
<td>Late infantile, Juvenile and Protracted</td>
<td>Similar to other late infantile variants, with slightly later onset</td>
</tr>
<tr>
<td>CLN6</td>
<td>CLN6 (Gao et al. 2002, Wheeler et al. 2002)</td>
<td>Transmembrane Protein (ER)</td>
<td>SCMAS</td>
<td>Late infantile</td>
<td>Similar to other late infantile variations, with slightly later onset</td>
</tr>
<tr>
<td>CLN7</td>
<td>MFSD8 channel (Siintola et al. 2007)</td>
<td>Membrane Protein</td>
<td>SCMAS</td>
<td>Late infantile (variant)</td>
<td>Seizures, psychomotor decline, myoclonus, loss of vision</td>
</tr>
<tr>
<td>CLN8</td>
<td>CLN8 (Ranta et al. 1999)</td>
<td>Transmembrane Protein (ER)</td>
<td>SCMAS</td>
<td>Late infantile (variant)</td>
<td>Epilepsy with progressive mental retardation, seizures, progressive mental deterioration, motor and behavioral problems (Mouse CLN8 -/- shows loss of vision)</td>
</tr>
<tr>
<td>CLN9</td>
<td>CLN9 (Schulz et al. 2004)</td>
<td>Unknown</td>
<td>SCMAS</td>
<td>Juvenile</td>
<td>Similar to other juvenile onsets</td>
</tr>
<tr>
<td>CTSD</td>
<td>Cathespsin D (Siintola et al. 2006b)</td>
<td>Soluble Enzyme</td>
<td>SAPs</td>
<td>Congenital and Late infantile</td>
<td>Congenital form of NCL</td>
</tr>
<tr>
<td>Unknown</td>
<td>Unknown (Siintola et al. 2006a)</td>
<td>Unknown</td>
<td>SAPs</td>
<td>Adult</td>
<td>Parry Disease, similar to other adult onset NCLs</td>
</tr>
</tbody>
</table>
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, Kytälä 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>
<thead>
<tr>
<th>Storage Disorder</th>
<th>Protein Defect</th>
<th>Storage Material</th>
<th>CNS Involved?</th>
<th>Clinical Presentation</th>
<th>Citations</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Mannosidosis</td>
<td>α-mannosidase</td>
<td>Mannose containing oligosaccharides</td>
<td>Yes</td>
<td>Behavior/learning difficulty, skeletal abnormality, immune deficiency, hearing impairment</td>
<td>(Desnick 2004, Crawley et al. 2006, Beck 2007)</td>
</tr>
<tr>
<td>Cystinosis</td>
<td>CTNS, cystine transporter</td>
<td>Intracellular cystine crystals</td>
<td>Yes, if untreated</td>
<td>Renal Fanconi Syndrome, renal glomerular failure, systemic complications</td>
<td>(Kleta and Gahl 2004)</td>
</tr>
<tr>
<td>Fabry</td>
<td>α-galactosidase</td>
<td>Globotriasylceramide (Gb3)</td>
<td>Yes</td>
<td>Multisystemic (involving heart, kidney and CNS), angiokeratoma</td>
<td>(Desnick 2004, Beck 2007)</td>
</tr>
<tr>
<td>Gaucher Type I</td>
<td>β-glucosidase</td>
<td>Glucocerebrosides</td>
<td>No</td>
<td>Hepatosplenomegaly, anemia, thrombocytopenia, skeletal complications</td>
<td>(Desnick 2004, Beck 2007)</td>
</tr>
<tr>
<td>Gaucher Type II</td>
<td>β-glucosidase</td>
<td>Glucocerebrosides</td>
<td>Yes</td>
<td>Acute neuropathy, severe visceral, hematological involvement, skeletal involvement, supranuclear gaze palsy, seizures</td>
<td>(Desnick 2004, Beck 2007)</td>
</tr>
<tr>
<td>Gaucher Type III</td>
<td>β-glucosidase</td>
<td>Glucocerebrosides</td>
<td>Yes</td>
<td>Chronic neuropathy, continuum of presentation found in Type II</td>
<td>(Desnick 2004, Beck 2007)</td>
</tr>
<tr>
<td>Hurler (a form of MPS I)</td>
<td>α-iduronidase</td>
<td>Dermatan sulphate and heparan sulphate</td>
<td>Yes</td>
<td>Skeletal disease, organomegaly, heart disease, mental retardation</td>
<td>(Desnick 2004, Futerman and van Meer 2004, Beck 2007)</td>
</tr>
<tr>
<td>Metachromatic Leukodystrophy</td>
<td>Arylsulfatase A</td>
<td>Sulfatide</td>
<td>Yes</td>
<td>Myelin degeneration in CNS and peripheral nervous system, progressive ataxia, seizures, quadriplegia, decerebration</td>
<td>(Desnick 2004, Biffi et al. 2006, Beck 2007)</td>
</tr>
<tr>
<td>Disorder</td>
<td>Enzyme</td>
<td>Substrates</td>
<td>Phenotype</td>
<td>References</td>
<td></td>
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<tr>
<td>----------------------------------</td>
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</tr>
<tr>
<td>Mucopolysaccharidosis I (MPS I)</td>
<td>α-iduronidase</td>
<td>Dermatan sulphate and heparan sulphate</td>
<td>In Hurler, may also include corneal clouding, joint contractions</td>
<td>Desnick 2004, Futerman and van Meer 2004, Beck 2007</td>
<td></td>
</tr>
<tr>
<td>Wolman</td>
<td>Acid lipase</td>
<td>Cholesterol esters and triglycerides</td>
<td>Progressive feeding intolerance, diarrhea, massive hepatosplenomegaly, liver cirrhosis, pulmonary infiltrates and enlarged adrenal glands with punctate calcification</td>
<td>Desnick 2004, Beck 2007, Stein et al. 2007</td>
<td></td>
</tr>
</tbody>
</table>
Enzyme activity would be needed. Purification of enzymes for ERT is used widely, requiring large amounts of enzyme. However, for ERT to be used broadly, large amounts of enzyme would be needed. Purification of enzymes 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 Mucopolysachharidosis 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 Mucopolysachharidosis. 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 myelin 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 α-LN2 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 sub-unit 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 I (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 adenovirus-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-associative 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 TPPI 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 TPPI 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-glucuronidase 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 Clenbuterol (β2-adrenoceptor agonist). Clenbuterol also enhances axon regeneration of lesioned motor neurons. However, the mechanism(s) whereby Clenbuterol 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 lysosomotropic 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 tumorogeneity, 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.

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REFERENCES


Perspectives on NCL therapies


