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Gene Therapy for Treatment of Monogenic Neurodegenerative Diseases

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A confluence of key advances in genomics and gene therapy vector technology has inspired a tremendous volume of research, conducted with the goal of realizing the potential of gene therapy for the treatment of diseases affecting the central nervous system (CNS). The recent approval of Zolgensma (2019), an adeno-associated virus serotype 9 (AAV9)-based gene therapy for treatment of young children with spinal muscular atrophy, provided a concrete example of how gene therapy can improve the lives of patients with devastating neurological diseases. Lysosomal storage disorders (LSDs) are a group of approximately 70 inherited inborn errors of metabolism, approximately two-thirds of which present as pediatric progressive neurodegenerative diseases, and therefore represent significant opportunity to benefit from gene therapy. Herein, we summarize key features of gene therapy, relevant aspects of lysosomal biology, and the continuing unmet medical need of LSDs that provide rationale for their use as a platform to investigate *in vivo* gene therapy for treatment of neurodegenerative diseases.

Lysosomal Biology

Lysosomes are acidic membrane-bound organelles most commonly recognized as the key intracellular sites in which large complex molecular substrates (glycosides, lipids, phospholipids, proteins, and nucleic acids) are broken down into their basic components and recycled back into the cytosol for use in energy metabolism and biosynthesis (La Cognata et al, 2020; Platt, 2018). A growing body of evidence indicates that in addition to this important catabolic function, lysosomes function in a wide variety of cellular processes, including nutrient sensing, energy metabolism, cellular homeostasis, cholesterol regulation, vesicle trafficking, calcium signaling, plasma membrane repair, autophagy, cellular growth, and immunology; and that at least some of these functions result from the coordinated transcriptional regulation of genes encoding proteins involved in lysosomal biogenesis, lysosomal function, and cell growth (La Cognata et al, 2020; Marques and Saftig, 2019; Platt, 2018).

Lysosomal catabolism is accomplished by the activity of approximately 60 unique acid hydrolases. These are soluble enzymes that readily bind and degrade soluble macromolecules such as glycoproteins and oligosaccharides. However, they require assistance from detergent-like membrane-perturbing proteins (sphingolipid activator proteins, SAPs) to bind lipophilic membrane-associated macromolecules such as gangliosides and glycosphingolipids (La Cognata et al, 2020). Transporter proteins that export metabolites out of the lysosome and ATP-dependent proton pumps that maintain the acidic environment required for optimal acid hydrolase activity reside in the lysosomal membrane.

Acid hydrolases are synthesized within the rough endoplasmic reticulum and undergo posttranslational modification in the Golgi apparatus (Sands and Davidson, 2006). An important modification made to the majority of the acid hydrolases is the addition of mannose -6-phosphate (the M6P "tag"), which binds to M6P receptors (M6PRs) on the surface of late endosomes to facilitate delivery of the tagged hydrolase to lysosomes, at which point it dissociates from M6PR under the acidic conditions within the lysosome. Importantly, some of the enzyme does not get delivered to the lysosomes and is instead secreted out of the cell. This secretory pathway has significance for the application of gene therapy to treat LSDs, as discussed later in this review.

Lysosomal Storage Diseases

Lysosomal storage disorders are inherited metabolic disorders in which lysosomal dysfunction results in gradual accumulation of undegraded substrates, cellular dysfunction, tissue damage, and death (La Cognata et al, 2020; Pinto e Vairo et al, 2020; Sands and Davidson, 2006). Individually, LSDs are rare diseases with global incidences typically ranging from 1 in 50,000 to 1 in 250,000 live births. Collectively, however, they are fairly common, with an estimated combined incidence of 1 in 4,000 to 1 in 7,000 live births (La Cognata et al, 2020; Pinto e Vairo et al, 2020; Platt, 2018).

Lysosomal storage disorders may be caused by mutations in genes encoding a variety of proteins involved in normal lysosomal function. However, the most common cause of LSDs is loss of function (LOF) mutations in the genes that encode the lysosomal acid hydrolases, resulting in little (typically <10% of normal) to no residual enzyme activity (Giuliani et al, 2018; La Cognata et al, 2020; Sands and Davidson, 2006). Similar to other genetic enzyme deficiencies, LSDs are mostly monogenic, autosomal recessive diseases (La Cognata et al, 2020; Platt, 2018; Sands and Davidson, 2006). The mechanisms by which substrate accumulation results in cellular dysfunction and ultimately cell death are not completely understood, but are thought to be related to the impact of accumulated substrates to disrupt cellular transport and degradation (eg, autophagy, endocytosis), calcium homeostasis, oxidative stress, cell death pathways, inflammation, and activation of microglia and macrophages (Giuliani et al, 2018; Platt, 2018). GM1 ganglioside (see "GM1 gangliosidosis" below) and other glycosphingolipids (GSLs) like galactosylceramide (see "Krabbe Disease" below) reside in membranes within lipid rafts – microdomains in which GSLs associate with lipids and proteins – that modulate multiple cell adhesion, signaling, and regulatory processes involved in neuronal differentiation, maturation, transmission, and protection (Platt, 2018). Disruption of microdomain integrity due to excessive accumulation of substrate contributes mechanistically to profound and progressive neurodegeneration.

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LSDs typically manifest as a continuum of disease ranging from patients with greater enzyme deficiency and earlier symptom onset, with more severe and more rapidly progressing disease, to patients with less severe enzyme deficiency, later symptom onset, and less severe, more slowly progressing disease. The level of residual enzyme activity is generally related to, but not reliably predictive of, age of onset and disease sever-

ity. Phenotypic variation may be attributed to a number of factors, including 1) the large number and distribution of causative mutations and their variable impact on substrate binding, catalytic activity, and/or enzyme stability (Deane et al, 2011; Ou et al, 2018); and 2) genetic or epigenetic modifiers which may, for example, explain observations of variable phenotypes among patients carrying the same mutation on the same genetic background, including monozygotic twins (Giuliani et al, 2018; Platt, 2018; Sands and Davidson, 2006). The lysosomal acid hydrolases are ubiquitously expressed and often have multiple substrates; thus, accumulation of substrates in the face of acid hydrolase deficiency depends on the tissue expression pattern of the substrates themselves and their relative affinities for the mutated hydrolase. The CNS is especially vulnerable to the effects of substrate accumulation. Concomitant substrate accumulation in peripheral tissues may occur depending on the particular LSD.

Fundamentals of *In Vivo* AAV-Based Gene Therapy for Treatment of Lysosomal Storage Disorders

Lysosomal storage diseases, especially those caused by mutations in the genes that encode the acid hydrolases, are considered excellent candidates for gene therapy because 1) they are monogenic disorders; 2) enzyme levels are generally less than 10% of normal reference levels, suggesting that overexpression of enzyme resulting in a range of increased enzyme activity may be sufficient to have therapeutic benefit (possibly even reverting the clinical phenotype); and 3) they are secreted enzymes taken up by neighboring cells via the M6PR pathway, thereby amplifying the effect of transducing a limited population of cells (a phenomenon referred to as "cross-correction" of cells) (La Cognata et al, 2020; Sands and Davidson, 2006).

Gene therapy takes advantage of the natural ability of viruses to infect cells to introduce a normal fully functional copy of a gene. Adeno-associated viruses (AAVs) are the most frequently used viruses for gene therapy (both approved and investigational) of a variety of diseases, including neurodegenerative diseases. They are naturally occurring nonreplicating viruses but do not themselves cause illness (Wang et al, 2019). They contain a single-stranded DNA genome surrounded by a protein shell (icosahedral protein capsid), the composition of which determines cellular tropism. Serotypes such as AAV1, AAV2 and AAV9 are used in neurodegenerative diseases (Wang et al, 2019).

Therapeutic recombinant AAV vectors are made by removing nearly all of the viral DNA and replacing it with a "gene expression cassette" that contains the functional therapeutic gene (the transgene) and certain regulatory elements necessary for proper transgene expression. Essentially, the only original AAV elements that remain are small pieces of DNA called inverted terminal repeats which are located on either end of the inserted gene expression cassette. These are important for proper packaging of the therapeutic gene into the AAV vector and for circularization of the functional gene expression cassette, which, once delivered to cells, persists in the nucleus as stable nonintegrating episomal DNA (Wang et al, 2019), allowing for potential long-term expression of the functional transgene in cells that are slowly or no longer dividing (eg, neurons). New functional protein is then made using the cell's normal machinery for transcribing DNA sequences.

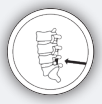
Direct Delivery of Gene Therapy Vectors to Cerebrospinal Fluid for Treatment of Neurodegenerative Disease: Suboccipital Puncture for Delivery to Cisterna Magna

Gene therapy trials for treatment of neurodegenerative diseases are ongoing using both intravenous and intrathecal vector administration. Intravenous administration of the vector is less invasive, but direct administration of vector into the cerebrospinal fluid (CSF) has several advantages over systemic delivery, including (Hinderer et al, 2020): 1) significantly lower dose requirements and less systemic toxicity; 2) higher levels of transduction of the CNS with more global biodistribution of vector; and 3) potentially less impact of pre-existing neutralizing antibodies to the AAV capsid (Table 1).

With respect to which delivery route results in optimal delivery of AAV vectors directly to CSF, large animal studies have demonstrated that, compared to lumbar puncture (LP), intracisterna magna (ICM) injection via suboccipital puncture was observed to be at least 10- to 100- fold more efficient in transducing the spinal cord and brain, respectively (Hinderer et al, 2014; Hinderer et al, 2020). Compared to intracerebroventricular (ICV) administration, ICM injection was associated with a lower risk of T-cell response to the transgene product and showed no evidence of the severe lymphocytic inflammation in the region surrounding the needle track and perivascular lymphocytic infiltration observed throughout the brain in animals injected by ICV (Hinderer et al, 2018).

Image-guided ICM delivery of gene therapy vectors has recently advanced to the clinical trial setting. Initial reports across a total of 11 unique patients ranging in age from 5 to 59 months and treated with AAV9-based

TABLE 1. Systemic and intrathecal routes of vector administration.

Location	 IV	 Lumbar	 Intra-Ventricular	 Cisterna Magna
Brain Delivery	Limited / Very High Doses	Limited	Diffuse	Diffuse
Toxicity	Systemic	Low	High	Low
AAV Antibody Impact	Limits efficacy	No Impact	No Impact	No Impact
Procedure	Routine	Routine	Interventional Radiologist	Interventional Radiologist or Neurosurgeon

Gurda et al, 2015; Hinderer et al, 2014, 2015, 2020; Pukeanas et al, 2021; Wang et al, 2019)

gene therapy for severe MPS II (Hunter's syndrome, n=8) (Nevoret et al, 2021; Pukenas et al, 2021), MPS I (n=1) (Wang et al, 2021), and Type 2 Gaucher disease (n=2) (Zibly et al, 2021) noted no procedure-related complications or serious adverse events. Although follow-up was variable across these studies, preliminary evidence of widely distributed transgene expression, directionally appropriate improvements in disease biomarkers, and both CNS and systemic efficacy suggest that ICM is a safe and effective route of administration for gene therapy (Nevoret et al, 2021; Wang et al, 2021; Zibly et al, 2021).

Lysosomal Storage Disorders as a Model for the Potential of Gene Therapy to Treat Monogenic Neurodegenerative Diseases

GM1 Gangliosidosis

GM1 gangliosidosis is caused by LOF mutations in the galactosidase beta1 (*GLB1*) gene, which encodes β-galactosidase (β-gal, E.C. 3.2.1.23), a lysosomal acid hydrolase that degrades GM1 ganglioside and other β-galactose-containing glycoconjugates including keratan sulfate and N- and O-linked oligosaccharides (Jarnes Utz et al, 2017; Lang et al, 2020; King et al, 2020). Patients with GM1 gangliosidosis have little (<10%) to no β-gal activity, resulting in progressive lysosomal accumulation of β-gal substrates, interference with a number of lysosomal signaling and metabolic functions, cellular apoptosis, and end-organ damage (Jarnes Utz et al, 2017; Lang et al, 2020). GM1 ganglioside is naturally abundant in neurons of the brain, and therefore accumulates predominantly in the brain of patients with GM1 gangliosidosis (Lang et al, 2020). Keratan sulfate and oligosaccharides accumulate primarily in peripheral tissues, including the eyes, liver, spleen, heart, and bone, and account for the peripheral manifestations of the disease (Lang et al, 2020).

Four clinical phenotypes have been described based on age at symptom onset and disease severity (Jarnes Utz et al, 2017; Lang et al, 2020; King et al, 2020; Regier et al, 2019):

In Early Onset Infantile (Type 1) GM1 gangliosidosis, symptom onset occurs by 6 months of age with profound hypotonia and neurodevelopmental delay in nearly all patients. Neurodegeneration progresses rapidly, with decerebrate rigidity, deafness, and blindness commonly observed by 1 year of age. Coarse facies, macular

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cherry-red spots, feeding difficulties, and dermatologic abnormalities, when present, may represent leading clinical signs. This is the most common (>60%) and most severe form of the disease. Disease presentation is relatively homogeneous, and progression follows a predictable course with death by early childhood. Late Onset Infantile (Type 2a) gangliosidosis has symptom onset between 6 months and 2 years of age and is notable for plateauing of motor and cognitive development followed by unrelenting developmental regression. Relative to early- onset infantile GM1 gangliosidosis, coarse facial features and macular cherry-red spots are less frequently reported; corneal clouding and seizures are more commonly reported. Overall, symptomology of the Late Onset Infantile phenotype is similar to that of Early Onset Infantile GM1 gangliosidosis, but with a slower rate of disease progression. Life expectancy typically is mid-to-late childhood prior to adolescence; some cases extending into adolescence or the teenage years are reported. Juvenile (Type 2b) gangliosidosis presents with symptoms

between 2 and 5 years of age. Disease presentation is more heterogeneous than the infantile phenotypes with respect to the age at onset of various symptoms, clinical features, and life expectancy. Dystonia leading to gait abnormalities, change in verbalization of words, and strabismus or abnormal eye movements have been noted as leading symptoms. Life expectancy is generally from late childhood prior to adolescence, yet some patients have lived into their teens or early adulthood. Adult (Type 3) gangliosidosis is the most heterogeneous and least severe of the phenotypes. This includes all patients with onset of disease at greater than 5 years of age, although symptoms typically present in the second or third decade. This phenotype is characterized by progressive neurological impairment, with cerebellar dysfunction, and dystonia; patients often present with limb-girdle weakness, followed by development of ataxia and progressive neuromuscular weakness, with eventual loss of independent ambulation. Slurred speech may be a leading sign. Life expectancy is highly variable, possibly extending to the third or fourth decade. There are currently no disease- modifying treatments for GM1 gangliosidosis. Current management is limited to supportive care, with focus on respiratory health, seizure management, and nutrition.

Krabbe Disease

Krabbe disease (globoid cell leukodystrophy) is caused by LOF mutations in the *GALC* gene, which encodes galactosylceramidase (galactocerebrosidase, also called GALC, E.C. 3.2.1.46). GALC enzyme is a lysosomal acid hydrolase that catabolizes glycolipids, including galactosylceramide (the major myelin lipid involved in normal turnover and maintenance of myelin) and galactosylsphingosine (psychosine, a highly cytotoxic lipid metabolite) (Bascou et al, 2018; Bradbury et al, 2020).

Patients with symptomatic Krabbe have very low (0% to 5%) residual enzyme activity. Psychosine accumulation consequent to galactocerebrosidase deficiency causes neuroinflammation, severe demyelination, axonopathy, and neuronal death (Bascou et al, 2018; Beltran-Quintero et al, 2019; Page et al, 2019). The myelin-producing oligodendrocytes in the CNS and Schwann cells in the peripheral nervous system (PNS) are particularly sensitive to the cytotoxic effects of psychosine. Myelin breakdown is accompanied by reactive astrocytic gliosis and infiltration of giant multinucleated macrophages ("globoid cells") to the CNS and PNS (Suzuki, 2003). Overall, the degradation within both the CNS and PNS manifests clinically as progressive neurodegeneration, spasticity, irritability, loss of vision and hearing, seizures, and premature death (Bascou et al, 2018).

Four clinical phenotypes have been described based on age at symptom onset and disease severity. Patients with Early Infantile Krabbe disease, which accounts for 60% to 70% of diagnoses, experience onset of symptoms before 6 months of age (symptoms may be present *in utero*). This is the most aggressive form of Krabbe disease, with rapid progression of neurological deficits and early mortality (Escolar et al, 2006; Duffner et al, 2011; Beltran-Quintero et al, 2019). Infants with Early Infantile Krabbe disease may present with extreme irritability and excessive crying, feeding difficulties, fisted hands, poor head control, stiffness, or arching. The disease course of Early Infantile Krabbe disease is highly predictable and severe, with rapid progression to include loss of acquired milestones, staring episodes, apnea, worsening peripheral neuropathy, severe weakness, unresponsiveness to stimuli, seizures, blindness, deafness, and death by 2 years of age (Duffner et al, 2011; Beltran-Quintero et al, 2019). Late Infantile Krabbe disease has traditionally been defined by first signs or symptoms between 6 to 36 months of age. Clinical presentation and disease course are more heterogeneous than the early infantile phenotype. Most common findings at presentation include loss of acquired developmental milestones, irritability, abnormal gait, motor delay, and abnormal muscle tone. Patients have progressive psychomotor regression, loss of vision and hearing, and seizures. Life expectancy is around mid-childhood (median age 7 years) (Bascou et al, 2018). Juvenile and Adult forms of Krabbe disease are less common, less well characterized, and more heterogeneous than the infantile forms. Symptom onset has been described as occurring between 3 years and 5 years of age (Komatsuzaki et al, 2019) or between 13 months to 10 years of age (Duffner et al, 2012). Children in the former cohort initially presented with gait abnormalities. Vision loss was not a common presenting sign. Disease progression most commonly included gait changes, fisting, spasticity, poor feeding, and hemiparesis. Life expectancy (reportedly 7 to >10 years old) generally correlated with the age of symptom onset. A recent report from a retrospective study of natural history data (n=248) demonstrated that 80% of juvenile patients were alive at 16 years and 88% of adult patients were alive at age 19 (Komatsuzaki et al, 2019). Disease progression is generally slower and the presentation more heterogeneous in the adult form of Krabbe disease compared to the juvenile form (Orsini et al, 2018).

Currently, hematopoietic stem cell therapy (HSCT, umbilical cord blood transplant, allogeneic peripheral blood stem cells, or allogeneic bone marrow) is the only treatment option for patients with Krabbe disease (Page et al, 2019). Treatment benefit has been observed in presymptomatic or mildly symptomatic patients, yet continuing progressive gross motor deficits (Escolar et al, 2005, Duffner et al, 2009) and other residual impairments (Duffner et al, 2009), and transient benefit in some patients indicate significant remaining unmet need. Restoration of brain GALC activity following HSCT requires months for transplanted cells to engraft, migrate to the CNS, differentiate, and restore normal microglial activity and enzyme levels. Once engrafted, newly synthesized GALC is secreted and taken up by other cells (Nagano et al, 1998), thus benefiting from cross-correction. But recent reports that HSCT influenced CNS-specific disease pathology without improving peripheral nerve disease (Wright et al, 2017; Allewelt et al, 2018) suggest that cross-correction does not occur to the degree sufficient to correct clinical deficits throughout both the CNS and PNS. Accordingly, despite progress with HSCT in treating patients with Krabbe disease, persistent unmet need indicates the search for new or additional cell and gene therapy options must continue. For patients who do not receive HSCT, disease management consists of supportive care, with focus on managing the gastrointestinal, respiratory, and neurological manifestations of the disease (Orsini et al, 2018).

Conclusion

The discovery of neurotropic AAV vectors coupled with advances in techniques for delivery of vectors directly to CSF has brought more clearly into focus the vision of providing long-lasting, potentially curative treatment of inherited diseases with single administration of a gene therapy vector. These advances are driving down the vector dose required to achieve the therapeutic objective, and theoretically reducing (although not yet eliminating) immunogenicity and toxicity, two persistent challenges of gene therapy. Lysosomal storage disorders, the majority of which are monogenic autosomal recessive diseases caused by missense mutations in genes that encode secreted acid hydrolases, provide an excellent platform for continued exploration of the potential of gene therapy to treat neurodegenerative diseases. To that end, a number of trials are ongoing (Pinto e Vairo et al, 2020; Pratt, 2018; clinicaltrials.gov) or are planned. In addition to providing treatment for the individual LSDs that are the focus of specific clinical trials, it is expected that ongoing clinical trials will provide new insights and technological advances to fuel continued improvements, with possible broader application to all neurodegenerative diseases.

Selection of the route of administration for delivery of gene therapy vectors is an important consideration. In diseases of the central nervous system for which broad biodistribution of vector is desirable, delivery of vector directly into the CSF has advantages over more invasive focal injection into the brain parenchyma or systemic delivery (IV). Image-guided delivery of vector via the cisterna magna has been demonstrated to be more efficient than LP injection and safer than ICV administration in non-human primates with brain size and anatomy similar to that of infants. Initial reports from a small cohort of patients from 5 to 59 months of age suggest that modern image-guided ICM injection under anesthesia may represent a safe and feasible vector delivery method.

An important obstacle that must be overcome to realize the full potential of gene therapy, or any other treatment modality, is more efficient and timely diagnosis of rare diseases. Unfortunately, the presence of symptoms indicates existing ongoing progressive disease. Therefore, early diagnosis is critical, especially in patients with early infantile LSD phenotypes who experience rapid neurodegeneration, and in the context of existing and emerging clinical trials that may represent a potential opportunity for investigational treatment. GM1 gangliosidisosis and Krabbe disease, like most rare diseases, are diagnosed ultimately via clinical assessment, and some combination of enzyme activity, mutation analysis, and substrate measurement (as appropriate). Significant delay to achieving proper diagnosis has been noted in both of these diseases as well as many of the LSDs. Newborn screening is of benefit in early identification of patients while there is a window of opportunity for intervention before irreversible neurological deterioration has occurred.

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