Enzyme Replacement Therapy for Lysosomal Diseases: Lessons from 20 Years of Experience and Remaining Challenges

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Abstract

In 1964, Christian de Duve first suggested that enzyme replacement might prove therapeutic for lysosomal storage diseases (LSDs). Early efforts identified the major obstacles, including the inability to produce large quantities of the normal enzymes, the lack of animal models for proof-of-concept studies, and the potentially harmful immune responses to the "foreign" normal enzymes. Subsequently, the identification of receptor-mediated targeting of lysosomal enzymes, the cloning and overexpression of human lysosomal genes, and the generation of murine models markedly facilitated the development of enzyme replacement therapy (ERT). However, ERT did not become a reality until the early 1990s, when its safety and effectiveness were demonstrated for the treatment of type 1 Gaucher disease. Today, ERT is approved for six LSDs, and clinical trials with recombinant human enzymes are ongoing in several others. Here, we review the lessons learned from 20 years of experience, with an emphasis on the general principles for effective ERT and the remaining challenges.

LYSOSOMAL STORAGE DISEASES AND THE RATIONALE FOR ENZYME REPLACEMENT THERAPY

The lysosomal storage diseases (LSDs) are a group of more than 50 disorders, most of which result from the deficient activity of a specific lysosomal enzyme and the progressive accumulation of its substrate(s), which include sphingolipids, glycogen, mucopolysaccharides, and glycoproteins. The characterization of the specific metabolic and genetic defects in these disorders has markedly increased our understanding of lysosomal biology, including enzyme targeting, intracellular transport, and the complex pathways involved in the degradation of macromolecules (for comprehensive reviews, see 22, 94, 95, 101).

The notion that LSDs could be treated by replacing the defective enzymes with their normal counterparts was first suggested by Christian de Duve (25) in 1964. Subsequent experiments demonstrated that when the appropriate active enzyme was added to the media of enzyme-deficient cultured fibroblasts from individuals with specific LSDs, the exogenous enzyme gained access to and degraded the substrates accumulated in the lysosomes (20, 89, 92). Notably, only 1%–5% of normal intracellular enzyme activity was required to correct the metabolic defects in the enzyme-deficient cells (31, 54, 74, 122). The subsequent discovery that lysosomal enzymes are targeted to the lysosome by the mannose-6-phosphate receptor–mediated pathway (for reviews, see 31, 54, 74, 122), along with the discovery that the mannose-6-phosphate receptors on the plasma membranes of cells mediate the cellular uptake and delivery of the intravenously administered normal enzymes to the lysosomes, provided further rationale for the treatment of nonneural LSDs by enzyme replacement therapy (ERT).

In addition, the fact that most LSDs have significantly milder subtypes with low levels of residual enzymatic activity also indicated that it was not necessary to restore full activity, or even heterozygous levels of activity, in the treated individuals, provided that the enzyme effectively reached the proper sites of pathology. Thus, these studies established the rationale for the early clinical studies of ERT in the LSDs. Here, we review the current status of ERT for LSDs and emphasize the principles for effective treatment and the remaining challenges. **Table 1** summarizes the LSDs for which ERT is approved or in clinical trials.

EARLY CLINICAL STUDIES OF ENZYME REPLACEMENT THERAPY

Beginning in the early 1970s, ERT pilot clinical studies were undertaken in several LSDs (Fabry, Gaucher, Pompe, and Sandhoff diseases) by intravenous infusion of the respective normal human enzyme. In each case, the partially purified enzyme was rapidly cleared from the circulation ($t_{1/2}$ of ~10–20 min), and there was evidence for clearance of the respective accumulated substrate(s) (for reviews, see 29, 31, 35, 109).

These early encouraging studies supported the feasibility of enzyme replacement. However, they also clearly indicated that the treatment of disorders with primary neuronal involvement was not feasible by this approach, because the intravenously administered enzymes did not cross the blood-brain barrier (64). Thus, investigators realized that ERT for disorders with severe neurologic involvement (such as Tay-Sachs, Sandhoff, and type A Niemann-Pick diseases) was not feasible, and focused their efforts on those without significant neurologic involvement.

In 1972 and 1979, international workshops on "enzyme therapy in genetic diseases" reviewed the developments in the area and identified the major obstacles confronting successful ERT in LSDs at the time (29, 31, 35, 109). These included (*a*) the inability to produce and purify sufficient quantities of lysosomal enzymes, including specific glycoforms; (*b*) the inability to target exogenously administered enzymes to specific tissue and cellular sites of pathology, particularly bones, cartilage,

Table 1 Characteristics of lysosomal storage diseases for which enzyme replacement therapy is approved or in clinical trials

Disease	Subtype	Deficient enzyme	Inheritance	Residual activity	Central nervous system involvement	Primary site(s) of pathology	Major manifestations	Animal models available
Fabry disease	Classic	α-Galactosidase A	XR	−	−	Vascular endothelium	Renal failure, pain, skin lesions, strokes	KOM
	Later onset	α-Galactosidase A	XR	+	−	Cardiomyocytes, renal cells	Cardiomegaly, renal failure	
Gaucher disease	Type 1	β-Glucocerebrosidase	AR	+	−	RES, bone	Hepatosplenomegaly, skeletal disease, pancytopenia	KIM
	Type 2	β-Glucocerebrosidase	AR	−	+	RES, neurons	Severe neurodegenerative disease, hepatosplenomegaly, death by age 2	
	Type 3	β-Glucocerebrosidase	AR	+	+	RES, neurons	Intermediate phenotype between types 1 and 2, neurodegenerative course, hepatosplenomegaly, pancytopenia, bone pain and fractures	
α-Mannosidosis	−	α-Mannosidase	AR	+/−	+	CTCs, RES, neurons, bone	Skeletal disease, mental retardation, corneal opacities, cataracts, hearing loss, organomegaly	C, KOM

(*Continued*)

Table 1 (*Continued*)

Disease	Subtype	Deficient enzyme	Inheritance	Residual activity	Central nervous system involvement	Primary site(s) of pathology	Major manifestations	Animal models available
MPS type I	Hurler syndrome	α-*L*-Iduronidase	AR	−	+	CTCs, RES, neurons, bone	Corneal clouding, skeletal disease, organomegaly, heart disease, mental retardation, death in childhood	C, D, KOM
	Hurler-Scheie syndrome	α-*L*-Iduronidase	AR	+	−	CTCs, RES, bone	Intermediate phenotype between the Hurler and Scheie subtypes	
	Scheie syndrome	α-*L*-Iduronidase	AR	+	−	CTCs, bone	Corneal clouding, stiff joints, normal intelligence and life span	
MPS type II	Severe	Iduronate-2-sulfatase	XR	−	+	CTCs, RES, neurons, bone	Skeletal disease, organomegaly, mental retardation, death by age 15; no corneal clouding	KOM
	Attenuated	Iduronate-2-sulfatase	XR	+	−	CTCs, RES, bone	Normal intelligence, short stature, survival to adulthood	
MPS type IV	Type A	*N*-Acetylgalactosamine-6-sulfatase	AR	?	−	CTCs, bone	Severe bone disease, hyperflexible joints, normal intelligence	KOM
MPS type VI	−	*N*-Acetylgalactosamine-4-sulfatase	AR	+	−	CTCs, RES, bone	Corneal clouding, severe skeletal disease, normal intelligence, organomegaly, cardiac involvement, survival to teens	C, D, KOM, R

Niemann-Pick disease	Type A	Acid sphingomyelinase	AR	−	+	RES, neurons	Severe neurodegenerative disease, hepatosplenomegaly, death by age 2	KIM, KOM
	Type B	Acid sphingomyelinase	AR	+	−	RES	Hepatosplenomegaly, pulmonary disease, pancytopenia, dyslipidemia	
Pompe disease	Infantile onset	Acid α-glucosidase	AR	−	−	Myocytes	Cardiomegaly, muscular weakness, hepatosplenomegaly, macroglossia	JQ, KOM
	Later onset	Acid α-glucosidase	AR	+	−	Myocytes	Juvenile onset: muscular weakness, respiratory difficulty, gastrointestinal abnormalities Adult onset: muscular weakness, slow progression	
Lysosomal acid lipase deficiency	Wolman disease	Lysosomal acid lipase	AR	−	−	Liver	Infantile onset, hepatosplenomegaly, steatorrhea, gastrointestinal symptoms, adrenal calcification, death within the first year of life	KOM
	CESD	Lysosomal acid lipase	AR	+	−	Liver	Later onset, hepatomegaly, cirrhosis, widespread lipid deposition	

Abbreviations: MPS, mucopolysaccharidosis; AR, autosomal recessive; XR, X-linked recessive; CESD, cholesteryl ester storage disease; CTC, connective tissue cell; RES, reticuloendothelial system. Animal models: C, cat; D, dog; JQ, Japanese quail; KIM, knock-in mouse; KOM, knockout mouse; R, rat.

and the central nervous system (CNS); and (*c*) the lack of animal models to enable evaluation of the pharmacokinetic and pharmacodynamic effects of enzyme administration.

ENZYME REPLACEMENT THERAPY IN TYPE 1 GAUCHER DISEASE: PROOF OF CONCEPT

In the 1980s, many researchers found the obstacles to ERT too formidable to pursue, and instead focused their research on more basic studies of lysosomal biology and disease. Only a few continued to perform clinical studies of ERT. Among these, Brady and colleagues (16) at the National Institutes of Health were determined to develop ERT for type 1 (non-neural) Gaucher disease, which is caused by the deficient activity of β-glucocerebrosidase (**Table 1**). The primary cellular site of pathology in Gaucher disease is the macrophage/monocyte system, and the bone marrow and reticuloendothelial organs of affected individuals become infiltrated with lipid-laden "foam" cells known as Gaucher cells. Patients develop massive enlargement of the liver and spleen, pancytopenia, and severe skeletal disease, resulting in bone pain and fractures.

Brady's group sought to reverse, or at least halt, the disease progression by using ERT with purified β-glucocerebrosidase from human placentae. Although most lysosomal glycoproteins are targeted to the lysosome via the mannose-6-phosphate receptor–mediated trafficking system, β-glucocerebrosidase is not [more recent studies revealed targeting via the Limp2 receptor (93)]. Therefore, to direct the enzyme to the macrophages, the *N*-linked oligosaccharide chains were modified by sequential removal of the sialic acid, β-galactosyl, and β-*N*-acetylglucosaminyl residues, thus exposing terminal mannose residues (37). This mannose-terminated form of the enzyme was efficiently recognized by the abundant mannose receptors on macrophage membranes and was then targeted to macrophage lysosomes for substrate catabolism (37). The early results of enzyme replacement using the mannose-terminated enzyme had encouraging but limited clinical effects, most likely due to the small doses administered (14). Subsequently, the Brady group reported that in these patients, intravenous infusions of large doses (2.0–3.0 mg kg^{-1}) of the mannose-terminated enzyme reduced the hepatosplenomegaly, improved hematological values, and led to substantial improvements in bone density as well as other manifestations (7, 8, 15). The reversal of lysosomal storage by ERT was unexpected, as most investigators presumed that the storage was irreversible and that at best, stabilization of the disease process would be obtainable. This demonstration that ERT was safe and well tolerated—and, most notably, that the enzyme could reverse years of substrate accumulation in these patients—provided the first clinical proof of concept for ERT in LSDs without primary neurologic involvement.

Initially, the β-glucocerebrosidase used for ERT (Ceredase®, developed by Genzyme Corporation) was purified from human placentae by industrial-scale techniques. Later, ERT with the mannose-terminated, recombinant human enzyme produced in Chinese hamster ovary (CHO) cells (Cerezyme®, also developed by Genzyme Corporation) was shown to be equally effective (50). Because an animal model for Gaucher disease did not exist, investigators experimented with the dose and dose schedule in patients to reduce the cost of therapy while still retaining therapeutic effectiveness. Eventually, it became appreciated that the clinical response was dose-dependent (27, 125) and that the maintenance dose was not significantly different from the dose originally used to reverse the years of substrate accumulation. Moreover, investigators found that 1.6 mg kg^{-1} (equal to 60 units kg^{-1}) every two weeks was more effective (and convenient) for type 1 Gaucher patients than more frequent administration of a lower dose. Also, the interruption of treatment resulted in substrate reaccumulation and reversal of the hematopoietic improvements (8). Because patients with type 1 Gaucher disease have residual enzymatic activity, the immunologic response to the normal enzyme

was not an issue (105). Administration of the enzyme in patients with the neurodegenerative type 2 or 3 Gaucher disease did not improve their neurologic manifestations, because the macromolecular enzyme could not cross the blood-brain barrier even at high doses.

The principles learned from the 20 years of experience with treating more than 5,500 type 1 Gaucher disease patients worldwide have included the following: (*a*) Enzyme delivery is receptor-mediated and dose-dependent, (*b*) substrate clearance is also dose-dependent, (*c*) years of substrate accumulation can be reversed in certain cells and organs, (*d*) a decreased dose or cessation of ERT results in substrate reaccumulation, (*e*) patients experience significant clinical benefit, and (*f*) ERT does not alter the progressive neurologic manifestations of the neuropathic subtypes. In addition, it soon became apparent that the earlier ERT is initiated, the more effective it is (even preventive), because some of the irreversible damage (e.g., bone disease, fibrosis) cannot be altered.

ADVANCES IN MOLECULAR BIOLOGY FACILITATE ENZYME REPLACEMENT THERAPY FOR LYSOSOMAL STORAGE DISEASES

The success of ERT in type 1 Gaucher disease stimulated investigators to develop and evaluate enzyme replacement for other LSDs (**Table 1**). **Table 2** shows the current status of diseases for which ERT is approved, pending approval, or in clinical trials. These efforts were facilitated in the 1980s and 1990s by the cloning of the cDNAs and genes encoding the human lysosomal enzymes, the development of eukaryotic overexpression systems to produce large quantities of the recombinant glycoprotein enzymes, and the use of gene-targeting techniques to generate knockout murine models for preclinical studies of ERT. These advances overcame two of the major early obstacles to ERT: the lack of sufficient amounts of human enzyme and the need for animal models for preclinical studies.

Production of Recombinant Human Enzymes

Although many proteins can be produced in large quantities in prokaryotic systems, it became obvious that even highly efficient prokaryotic systems were not useful for the expression of lysosomal enzymes because they did not carry out the posttranslational modifications (e.g., *N*-linked glycosylation and mannose phosphorylation) needed for lysosomal enzyme stability, synthesis, and/or activity. Researchers therefore turned to CHO, human fibrosarcoma, and other cells that would perform the required posttranslational modifications to manufacture these enzymes.

Most human recombinant lysosomal enzymes were made in CHO cells because they are easy to grow and perform posttranslational modifications that are nearly identical to those of human cells. Importantly, it was discovered not only that the overexpression of a lysosomal cDNA in CHO cells resulted in the delivery of the encoded recombinant human enzyme to the lysosomes, but also, more importantly, that the majority of the recombinant enzyme was selectively secreted into the culture media (62), thereby facilitating large-scale production of the critical, highly glycosylated enzyme. Of note is that the secreted enzymes retained their terminal mannose-6-phosphate and sialic acid residues, whereas within the lysosomes the enzyme's oligosaccharide chains were trimmed by glycosidases. Other expression systems using human fibrosarcoma cells, transgenic animals, egg whites, and plant cells are also now being used to produce therapeutic human lysosomal enzymes.

Generation of Mouse Models for Lysosomal Storage Diseases and Preclinical Studies of Enzyme Replacement Therapy

In addition to the small number of naturally occurring animal models that had been identified for LSDs (55), the development of gene-targeting technology in the 1990s led investigators to generate many mouse

Table 2 Current status of enzyme replacement therapy for lysosomal storage diseases

Disease	Subtype(s)	Recombinant enzyme along with generic and/or trade name	FDA/EMA approval or clinical trial status	Approved/recommended dose	
				Administered dose and schedule	Total monthly dose
FDA/EMA approved					
Gaucher disease	Type 1	β-Glucocerebrosidase: imiglucerase (Cerezyme®, Genzyme Corporation)	Approved in 1991 (US) and 1997 (EU)	1.6 mg kg^{-1} biweekly	3.2 mg kg^{-1}
		β-Glucocerebrosidase: velaglucerase alfa (VPRIV®, Shire HGT)	Approved in 2010 (US and EU)	1.6 mg kg^{-1} biweekly	3.2 mg kg^{-1}
Fabry disease	Both classic and later onset	α-Galactosidase A: agalsidase beta (Fabrazyme®, Genzyme Corporation)	Approved in 2001 (EU) and 2003 (US)	1.0 mg kg^{-1} biweekly	2.0 mg kg^{-1}
		α-Galactosidase A: agalsidase alfa (Replagal®, Shire HGT)	Approved in 2001 (EU)	0.2 mg kg^{-1} biweekly	0.4 mg kg^{-1}
MPS type I	Hurler, Hurler-Scheie, and Scheie syndromes	α-*L*-Iduronidase: laronidase (Aldurazyme®, BioMarin Pharmaceutical/Genzyme Corporation)	Approved in 2003 (US and EU)	0.58 mg kg^{-1} weekly	2.32 mg kg^{-1}
MPS type II	Both severe and attenuated	Iduronate-2-sulfatase: idursulfase (Elaprase®, Shire HGT)	Approved in 2006 (US) and 2007 (EU)	0.5 mg kg^{-1} weekly	2.0 mg kg^{-1}
MPS type VI	—	*N*-Acetylgalactosamine-4-sulfatase: galsulfase (Naglazyme®, BioMarin Pharmaceutical)	Approved in 2005 (US) and 2006 (EU)	1.0 mg kg^{-1} weekly	4.0 mg kg^{-1}
Pompe disease	Infantile onset	Acid α-glucosidase: alglucosidase alfa (Myozyme®, Genzyme Corporation)	Approved in 2006 (US and EU)	20 mg kg^{-1} biweekly	40 mg kg^{-1}
	Later onset (juvenile and adult)	Acid α-glucosidase: alglucosidase alfa (Lumizyme®, Genzyme Corporation)	Approved in 2010 (US)	20 mg kg^{-1} biweekly	40 mg kg^{-1}
Ongoing clinical trials					
α-Mannosidosis	—	α-Mannosidase (Lamazym®, Zymenex)	Phase 2 trial completed in 2012	—	—
Gaucher disease	Type 1	β-Glucocerebrosidase: taliglucerase alfa (Uplyso®, Protalix Biotherapeutics)[a]	Phase 3 trial completed in 2011	1.6 mg kg^{-1} biweekly	3.2 mg kg^{-1}
Lysosomal acid lipase deficiency	Wolman disease	Lysosomal acid lipase (Synageva BioPharma)	Phase 1 trial to begin in 2012	—	—
	CESD	Lysosomal acid lipase (Synageva BioPharma)	Phase 1/2 trial completed in 2012	—	—
MPS type IV	Type A	*N*-Acetylgalactosamine-6-sulfatase: GALNS (BioMarin Pharmaceutical)	Phase 3 trial enrolling in 2012	—	—
Niemann-Pick disease	Type B	Acid sphingomyelinase (Genzyme Corporation)	Phase 2 trial to begin in 2012	—	—

Abbreviations: FDA, Federal Drug Administration; EMA, European Medicines Agency; CESD, cholesteryl ester storage disease; MPS, mucopolysaccharidosis.

[a]At the proof stage of this review, taliglucerase alfa (β-glucocerebrosidase, Uplyso®, Protalix Biotherapeutics) was approved by the FDA.

models, most of which had at least some of the biochemical, pathological, and/or clinical manifestations of their human counterparts. Thus, by the mid-1990s LSD researchers had new methods that provided large quantities of recombinant enzymes and disease-specific animal models to vigorously pursue the development and evaluation of ERT. Importantly, preclinical studies in animal models permitted evaluation of the pharmacokinetics and pharmacodynamics of ERT for LSDs. Because the Gaucher disease knockout mouse was not viable, the first such preclinical studies of ERT to demonstrate proof of concept were conducted in Fabry and type A Niemann-Pick knockout mice and in mucopolysaccharidosis (MPS) VII mice in the mid-1990s (63, 81, 97).

LYSOSOMAL CANDIDATES FOR ENZYME REPLACEMENT THERAPY: THERAPEUTIC CONSIDERATIONS

Several factors influence the selection of candidate LSDs for ERT. These include the target sites of pathology, the likelihood of reversing certain manifestations (e.g., reticuloendothelial system, skeletal, and neural diseases), and the presence or absence of residual enzymatic activity.

Biodistribution of Exogenously Administered Enzymes

Animal model and clinical studies have revealed organ-specific variations in response to ERT. The variable organ response is primarily due to the biodistribution of the infused enzymes and the relative density of the lysosomal receptors (e.g., mannose-6-phosphate, Limp2) on different cell types. Indeed, in the animal models it was found that for most recombinant lysosomal enzymes the biodistributions following intravenous injection were similar (e.g., 24, 63, 81, 97), with good distribution to the reticuloendothelial system and poor uptake by the brain and bones. Other clinically relevant organs (e.g., the kidneys in Fabry disease, the lungs in Niemann-Pick disease) received relatively small amounts of enzyme (63, 81). Notably, in the animal models, the tissue distribution of the intravenously infused enzymes and the amount and duration of substrate clearance (i.e., pharmacodynamics) from target sites of pathology were also dose-dependent (e.g., 24, 63, 81, 97).

Table 3 lists the tissue sites of pathology in some human LSDs that are easy or hard to reach based on the biodistribution and uptake of intravenously administered enzymes in the LSD animal models. As noted above, for each disease the infused enzymes must be delivered to specific and unique cell types, which explains why ERT is more effective for some LSDs than for others. For example, in type 1 Gaucher disease the major pathological cell type is the easily targeted macrophage; however, treatment must begin early to influence the progressive bone disease. Furthermore, ERT did not reverse the neurologic manifestations in patients with type 2 or 3 Gaucher disease (115). In Fabry

Table 3 Easy- and hard-to-reach tissues for in vivo delivery of intravenously administered enzymes

Disease	Subtype(s)	Easy to reach	Hard to reach
Gaucher disease	Type 1	Spleen, liver, bone marrow	Bone
	Types 2 and 3	Spleen, liver, bone marrow	Bone, brain
Fabry disease	Both classic and later onset	Vascular endothelium	Kidney, heart
Mucopolysaccharidoses	All	Spleen, liver, bone marrow	Bone, brain, cartilage
α-Mannosidosis	—	Spleen, liver, bone marrow	Bone, brain
Niemann-Pick disease	Type B	Spleen, liver, bone marrow	Alveolar macrophages
Pompe disease	Infantile onset	—	Heart, smooth muscle, skeletal muscle
	Later onset	—	Smooth muscle, respiratory skeletal muscle

disease, the major site of pathology is the vascular endothelium, which is readily accessed by exogenous enzymes, whereas the heart and kidneys take up <1% of the administered enzyme. In the MPSs, the severe bone and joint abnormalities result from defects in connective tissue cells (e.g., chondrocytes), which take up little if any of the intravenously administered enzyme.

As noted above, even for diseases with the same enzyme deficiency, certain clinical subtypes may be more amenable to ERT than others—e.g., type 1 Gaucher disease but not type 2 or 3. Another example is MPS type I: The severe subtype, Hurler syndrome (MPS IH), results in early-onset skeletal and neurologic manifestations, whereas the Hurler-Scheie (MPS IH-S) and Scheie (MPS IS) subtypes have manifestations that are more attenuated, characterized by later onset and the absence of mental retardation. Thus, the MPS IH-S and IS subtypes are more amenable to ERT than the MPS IH subtype. Clearly, the effectiveness of ERT in LSDs depends both on the delivery of sufficient amounts of the administered enzyme to the specific target sites of pathology and on the reversibility of certain clinical manifestations.

Immunologic Response to Enzyme Replacement Therapy

The absence or presence of the mutant enzyme protein [i.e., cross-reactive immunologic material (CRIM) negative or positive, respectively] in patients with LSDs primarily determines the immunologic response to ERT (see **Table 4**). In type 1 Gaucher disease, all patients have residual β-glucocerebrosidase activity (<10% of normal), and experience with more than 5,500 treated patients has documented that fewer than 15% of these individuals raise immunoglobulin G (IgG) antibodies against the normal enzyme (105); these antibodies have no measurable effect on efficacy (i.e., are nonneutralizing) and rarely cause infusion-associated reactions. In contrast, the majority of patients with classic Fabry disease, infantile-onset Pompe disease, type 2 or 3 Gaucher disease, and the severe forms of MPS I, II, and VI—all of whom have essentially no residual enzyme activity—develop IgG antibodies (see **Table 4**), typically after four to eight infusions (23, 52, 84, 86). These patients may also experience infusion-associated reactions including chills, rigors, and/or fevers, which do not markedly affect efficacy and can be managed conservatively by premedication with nonsedating antihistamines and antipyretics and by slowing the infusion rate, because these reactions are directly related to protein load (see **Table 4**). Importantly, patients who seroconvert decrease their antibody titers with time and may eventually develop tolerance to the recombinant enzyme. In rare instances, an LSD patient will raise IgE antibodies and have a life-threatening anaphylactic reaction. These patients require special treatment to induce tolerance (see Pompe Disease, below) (90).

In some patients, especially those who are CRIM negative and have high antibody titers, the IgG antibodies may neutralize a portion of the infused recombinant enzyme activity and/or block the mannose-6-phosphate moieties, resulting in decreased lysosomal delivery and/or substrate catabolism (e.g., 6, 28, 90). In infantile-onset Pompe disease, in which high doses of enzyme are administered (20 mg kg^{-1} biweekly), CRIM-negative and some CRIM-positive patients may develop high antienzyme antibody titers (>1 in 200,000), which may reverse the initial clinical improvement (88, 114). The antigen-IgG-antibody complex may be taken up into cellular lysosomes via the Fc receptor, which will be taken up primarily by macrophages.

CURRENT STATUS AND CHALLENGES OF ENZYME REPLACEMENT THERAPY

As indicated in **Table 2**, ERT is approved in the United States and Europe for six LSDs: type 1 Gaucher disease; Fabry disease; MPS I, II, and VI; and Pompe disease. Clinical trials also are under way for several others,

Table 4 Recommended doses, infusion-associated reactions, and antibody formation of human recombinant enzymes used to treat lysosomal storage disorders

Disease	Subtype(s)	Recombinant enzyme along with generic and trade name	Approved/recommended dose		Proportion of patients with infusion-associated reactions	Proportion of patients with IgG antibody formation
			Administered dose and schedule	Total monthly dose		
Gaucher disease[a]	Type 1	β-Glucocerebrosidase: imiglucerase (Cerezyme®, Genzyme Corporation)	1.6 mg kg^{-1} biweekly	3.2 mg kg^{-1}	13.8%	15%
		β-Glucocerebrosidase: velaglucerase alfa (VPRIV®, Shire HGT)	1.6 mg kg^{-1} biweekly	3.2 mg kg^{-1}	52%	1.9%
Fabry disease	Both classic and later onset	α-Galactosidase A: agalsidase beta (Fabrazyme®, Genzyme Corporation)	1.0 mg kg^{-1} biweekly	2.0 mg kg^{-1}	50%–55%	68%
		α-Galactosidase A: agalsidase alfa (Replagal®, Shire HGT)	0.2 mg kg^{-1} biweekly	0.4 mg kg^{-1}	52%	64%
MPS type I	Hurler-Scheie and Scheie syndromes	α-*L*-Iduronidase: laronidase (Aldurazyme®, BioMarin Pharmaceutical/Genzyme Corporation)	0.58 mg kg^{-1} weekly	2.32 mg kg^{-1}	32%	97%
MPS type II	Both severe and attenuated	Iduronate-2-sulfatase: idursulfase (Elaprase®, Shire HGT)	0.5 mg kg^{-1} weekly	2.0 mg kg^{-1}	15%	47%
MPS type VI	—	*N*-Acetylgalactosamine-4-sulfatase: galsulfase (Naglazyme®, BioMarin Pharmaceutical)	1.0 mg kg^{-1} weekly	4.0 mg kg^{-1}	54.5%	97%
Pompe disease	Infantile onset	Acid α-glucosidase: alglucosidase alfa (Myozyme®, Genzyme Corporation)	20 mg kg^{-1} biweekly	40 mg kg^{-1}	51%	95%
	Later onset (juvenile and adult)	Acid α-glucosidase: alglucosidase alfa (Lumizyme®, Genzyme Corporation)	20 mg kg^{-1} biweekly	40 mg kg^{-1}	≥5%	100%

Abbreviation: MPS, mucopolysaccharidosis.

[a]At the proof stage of this review, taliglucerase alfa (β-glucocerebrosidase, Uplyso®, Protalix Biotherapeutics) was approved by the FDA at an administered dose of 1.6 mg kg^{-1} biweekly and a total monthly dose of 3.2 mg kg^{-1}.

including α-mannosidosis, lysosomal acid lipase deficiency, MPS IVA (*N*-acetylgalactosamine-6-sulfatase deficiency, known as Morquio syndrome), and type B Niemann-Pick disease. A brief summary of the status of each of the approved ERTs and those in clinical trials is provided below, with an emphasis on the challenges for each and current strategies to overcome them.

Gaucher Disease

As noted above, type 1 Gaucher disease was the first LSD for which ERT was approved (1991) by the US Food and Drug Administration (FDA). The subsequent 20 years of experience have taught investigators many lessons, including the importance of dose, the reversibility of substrate accumulation in the macrophage/monocyte system, the lack of biodistribution to bone, and the inability of the infused enzyme to cross the blood-brain barrier for treatment of the neuropathic subtypes. In type 1 Gaucher patients, ERT has proven extremely effective and even preventive when initiated early in the disease course (124). Also, the discoveries that the plasma activity of chitotriosidase and the level of chemokine CCL18/PARC are indicators of macrophage activation and disease severity have led to the use of these molecules as biomarkers for monitoring therapy, and have demonstrated the importance of biomarkers for developing and monitoring LSD therapies in general (12, 60).

In addition to the mannose-terminated recombinant human β-glucocerebrosidase produced in CHO cells (imiglucerase, trade name Cerezyme®, Genzyme Corporation), two other enzyme preparations have been recently evaluated in type 1 Gaucher patients: velaglucerase alfa (VPRIV®, Shire HGT), which is produced in human fibrosarcoma cells (133) and was recently FDA approved, and taliglucerase alfa (Uplyso®, Protalix Biotherapeutics), which is produced in carrot cells (134) and was also recently FDA approved. Head-to-head clinical trials evaluating these products alongside Cerezyme have not been carried out.

In terms of the remaining challenges for Gaucher disease, treatment of the neuronopathic subtypes and improved treatment of bone disease remain the two most important obstacles. For the neuronopathic subtypes, it does not appear that high-dose therapy or early intervention will improve the neurologic disease; therefore, alternative therapies using small molecules that cross the blood-brain barrier or direct delivery of enzymes to the CNS are needed. For bone disease, it appears that early intervention may modify the ERT response, and combination therapies that target secondary storage materials or pathological pathways, or that improve the efficacy of enzyme delivery, may prove important (see below).

Fabry Disease

Fabry disease is an X-linked disorder resulting from the deficient activity of α-galactosidase A (α-Gal A) and the progressive lysosomal accumulation of its substrate globotriaosylceramide (GL-3). In classically affected males, who have no detectable α-Gal A activity, GL-3 accumulation in the vascular endothelium causes the major disease manifestations (33, 101). Clinical onset in affected boys includes severe acroparesthesias, angiokeratoma, hypohidrosis, and corneal/lenticular opacities. With advancing age, the progressive lysosomal GL-3 accumulation—particularly in the microvasculature—leads to renal failure, heart disease, strokes, and premature demise, typically in the fourth or fifth decade. Males with the later-onset subtype have residual α-Gal A activity and no vascular endothelial involvement. These individuals usually develop renal failure and/or heart disease in adulthood.

ERT was evaluated for Fabry disease in α-Gal A knockout mice, which provided the first information on the biodistribution, organ uptake, and substrate clearance of an intravenously administered lysosomal enzyme at different doses (110). Subsequently, ERT was developed in Fabry patients using recombinant human α-Gal A preparations produced in CHO cells (agalsidase beta, Fabrazyme®,

Genzyme Corporation) and in human fibrosarcoma cells (agalsidase alfa, Replagal®, Shire HGT) (4, 44, 100). Both Fabrazyme and Replagal were approved by the European Medicines Agency (EMA) in the European Union, but only Fabrazyme is approved by the FDA in the United States (30). Several studies comparing the two products' specific activity, biochemical composition, and cell uptake in fibroblasts and knockout Fabry mice have found that the enzymes have essentially the same specific activities and kinetic properties and similar glycosylation, although Fabrazyme has more mannose-6-phosphate and greater sialylation (76, 96). In vivo administration of the two enzymes to Fabry mice at the same dose indicated that Fabrazyme has greater uptake in the kidney and heart (76, 96), consistent with its higher mannose-6-phosphate content. At the FDA- and EMA-approved doses, Fabrazyme is administered at five times the dose of Replagal (**Table 2**).

The safety and effectiveness of ERT with Fabrazyme have been evaluated by two multicenter, multinational, randomized, double-blind, placebo-controlled clinical trials involving 58 and 82 patients, respectively (4, 44, 127). Fabrazyme was shown to clear the accumulated GL-3 in the vascular endothelium of the kidney, heart, and skin and to normalize the plasma GL-3 level (44, 111). The phase 4 Fabrazyme clinical trial demonstrated that even patients with advanced disease (serum creatinine between 1.2 and 3.0 mg%), when treated at 1.0 mg kg^{-1} biweekly, had slower progression than those in a matched placebo group (4). The effectiveness of ERT with Fabrazyme in stabilizing renal disease, improving cardiac involvement, and decreasing the extremity pain and gastrointestinal manifestations has also been reported in large registries, small cohort studies, and recent expert reviews (e.g., 98, 123).

Following a dose-ranging study from 0.07 to 0.1 mg kg^{-1}, which did not show a dose effect, Replagal was evaluated at 0.2 mg kg^{-1} biweekly in two single-site, randomized, double-blind, placebo-controlled studies (61, 100). In the pivotal registration study, which randomized 26 male patients, pain was the primary endpoint, and the enzyme's effect on renal function was evaluated (100). The FDA advisory committee did not accept the Replagal data for pain or renal function improvement (30). A subsequent study randomized 15 male patients to assess the enzyme's effect on cardiac involvement (61). In the latter study, left ventricular mass was decreased after six months compared with that in placebo-matched patients; however, the primary endpoint, reduction in heart biopsy GL-3 levels, did not achieve significance.

ERT dose in Fabry disease has been the subject of much discussion because the approved Replagal dose is the lowest of all ERTs for the LSDs (**Table 2**). To date, there have been no head-to-head randomized, double-blind trials of patients matched for sex, age, and severity to directly compare the effectiveness of Fabrazyme and Replagal on tissue substrate clearance and clinical outcomes at their approved doses of 1.0 mg kg^{-1} biweekly for Fabrazyme and 0.2 mg kg^{-1} biweekly for Replagal. Comparison of the published clinical studies is difficult because the disease spectrum is wide, there are no common mutations, and clinical variation occurs even in affected brothers. In addition, many studies have combined data from more severely affected classical males and milder later-onset males, and have often combined affected males and heterozygous females. Comparison of reports from the disease registries is also extremely difficult to evaluate (59). Thus, the evidence for effectiveness remains based on the randomized, double-blind, placebo-controlled studies for each individual drug (4, 44, 100, 111).

Recognizing these limitations, investigators recently carried out two clinical studies of the two drugs, both administered at 0.2 mg kg^{-1} biweekly, which did not reduce left ventricular mass, glomerular filtration rate, pain, or levels of substrate in plasma or urine; both drugs also raised antienzyme antibodies in affected males (118). Thus, at the same dose, the drugs had similar effects.

In Fabry disease, most classically affected males who have essentially no enzyme activity raise IgG antibodies to the infused enzymes,

whereas later-onset males and most heterozygotes do not (118). In an analysis of more than 700 males and females treated with Fabrazyme (who were not subclassified by classic or later-onset phenotype), 73% of males and 12% of females (68% overall) developed IgG antibodies (128). The effect of the antibodies on ERT has been studied by determining cardiac mass and urinary substrate levels, which indicated that high antibody titers can impact the effectiveness of substrate clearance. Of note is that antibody-positive patients treated with Fabrazyme at 1.0 mg kg^{-1} biweekly had persistently decreased urinary GL-3 levels and decreased heart mass, whereas those treated at 0.2 mg kg^{-1} biweekly with Fabrazyme or Replagal did not (117).

A novel approach to avoid raising antibodies against recombinant α-Gal A was to modify the highly homologous human enzyme α-*N*-acetylgalactosaminidase (also known as α-Gal B) so that it would hydrolyze GL-3 and related α-Gal A substrates (110). This enzyme engineering approach succeeded in creating a sheath enzyme; however, its kinetic properties required large amounts of infused enzyme to achieve the level of α-Gal A effectiveness in the Fabry mouse model.

The importance of early diagnosis and treatment of the LSDs has also been emphasized in Fabry disease, especially in the phase 4 trial in patients with advanced disease (4). Early treatment of classically affected males should begin in childhood when the first symptoms occur (or even before, for optimal results), as recommended by expert panels (32, 43). Recently, renal biopsies from affected boys demonstrated significant GL-3 accumulation—particularly in the podocytes, where it was reduced with 1.0 mg kg^{-1} biweekly but not with 0.2 mg kg^{-1} biweekly (87)—that was subsequently cleared with ERT, suggesting that early intervention may even be preventive (M. Mauer, personal communication). Efforts to identify affected males by newborn screening have been reported from Italy (104) and Taiwan (21, 22), and pilot studies are currently under way in Washington State and Illinois. The incidence of affected males with the classic subtype in Italy and Taiwan was 1 in ~37,000 and 1 in ~28,000, respectively, whereas the later-onset subtype was at least 10 times more frequent in each study (21, 22, 104). The challenge in the future will be to determine how early to start ERT in classically affected children and later-onset adults.

Another challenge is the fact that some heterozygous females with the classic subtype develop cardiac and/or renal disease (26, 42, 101, 129), presumably due to the skewing of random X inactivation. The difficulty is in predicting which heterozygotes will develop these manifestations, as biomarkers that reliably predict such individuals have not been identified, and thus continual monitoring of the heterozygotes is required to detect early signs of renal or cardiac involvement.

The Mucopolysaccharidoses

The MPSs comprise 11 distinct lysosomal enzyme deficiencies that have been clinically delineated into 7 types (82). ERTs are available for 3 of these disorders (MPS I, Hurler, Hurler-Scheie, and Scheie subtypes; MPS II, or Hunter syndrome; and MPS VI, or Maroteaux-Lamy syndrome), and are under development for several others (113). Unique to the MPSs is the fact that the enzymes are each involved in glycosaminoglycan (GAG) degradation, and therefore the patients present with severe connective tissue disease, particularly in the skin, trachea, joints, and bones. In addition, most MPS disorders have CNS involvement, with the exception of types IH-S, IS, IVA, and VI.

On the basis of prior animal model studies (24, 48, 65), pivotal multisite, multinational, randomized, double-blind, placebo-controlled clinical trials documented the clinical benefit of ERT for MPS I (130), MPS II (83, 84), and MPS VI (52) (**Table 2**). ERT for these disorders has provided several useful and important lessons. In general, these therapies reduce the reticuloendothelial cell storage of GAGs, leading to reduced organomegaly, increased mobility and breathing, and reduced pain in the treated patients. Joint mobility is also slightly

improved. It is also clear from this experience that the intravenously administered enzymes do not effectively reach the bone growth plates, articular cartilage, or CNS (**Table 3**). The improvements in joint mobility observed in some patients are likely due to soft tissue changes and reduction in inflammation (see below) rather than delivery of the enzymes to the joint cartilage.

One unique feature of the MPS diseases is that many of the patients undergoing ERT have also received hematopoietic stem cell transplants (HSCTs), which until recently were the only available treatment option for patients with these disorders (28). For example, bone marrow transplants for these diseases have been undertaken for more than three decades, and hundreds of patients have been transplanted. Most of this experience is in MPS I and II, with fewer transplants in the other MPS types. For severely affected MPS IH patients with CNS involvement, HSCT remains the treatment of choice because the intravenously administered enzyme cannot cross the blood-brain barrier (28). Transplantation has been shown to preserve intellectual development when performed early in the course of the disease, and is indicated for MPS IH patients under the age of 2 (see below). However, this procedure does carry morbidity and mortality risks, which have improved over time but are still considerable. Of interest is that ERT is increasingly being used as an adjuvant treatment before HSCT to improve the pretransplant condition (49).

Successful engraftment of bone marrow cells in MPS patients means that in addition to the systemically administered enzymes from ERT, these patients also have a continuous low-level release of enzymes from the transplanted bone marrow cells themselves, both systemically and locally at sites of pathology. Although ERT and HSCT are now frequently used in combination to treat MPS patients, there have been few studies to evaluate the additive benefits of the two treatments. In 2011, a consensus statement regarding the use of both ERT and HSCT in patients with MPS I was reported (28). It was agreed that (*a*) the preferred treatment for MPS IH patients diagnosed before age 2.5 remains HSCT; (*b*) in individual patients with an intermediate MPS IH-S phenotype, HSCT may be considered if there is a suitable donor, although there are no data on the efficacy of HSCT in patients with this phenotype; (*c*) all MPS I patients, including those who have not been transplanted or whose graft has failed, may benefit significantly from ERT; and (*d*) ERT should be initiated at diagnosis and may be of value in patients awaiting HSCT.

Another important lesson of ERT that has emerged from experiences in the MPS disorders relates to the treatment of neurologic disease. Animal model studies, including those in MPS VII mice and MPS I dogs, have suggested that the use of very high doses of intravenous enzymes very early in life (presymptomatic) could reduce GAG storage in the CNS and partially improve brain disease (120, 121). This approach has not been studied in humans, and at present MPS patients are treated only at the time of first clinical diagnosis, with enzyme doses (0.58–1.0 mg kg^{-1} weekly; **Table 2**) that have been shown to improve nonneurologic endpoints but are much lower than those used in the animal model studies. Therefore, under these conditions the systemically administered enzymes have not been effective at treating or even stabilizing the CNS complications of these disorders.

An alternative to high-dose systemic ERT for the CNS component of the LSDs that has been pioneered in the MPS disorders is intrathecal and/or intraventricular administration of the enzymes (2, 19, 36, 119). This has been studied in several MPS animal models and in a very limited number of MPS patients. For example, a recent animal study showed that intracerebroventricular and lumbar intrathecal administration of recombinant iduronate-2-sulfatase (the enzyme deficient in MPS II) in dogs and nonhuman primates results in widespread enzyme distribution in the brain parenchyma, including in the lysosomes of both neurons and oligodendrocytes (19). Lumbar intrathecal administration also resulted in enzyme delivery to the spinal cord,

where small amounts of enzyme were detected after intraventricular administration. Another recent study in MPS I cats showed that repeated intrathecal injection of recombinant human α-*L*-iduronidase reduced GAG storage to normal levels in the brain and, most important, showed that the storage material did not reaccumulate for up to 1 month after the last injection (119). These results suggested the potential of intrathecal enzyme dosing every 2–3 months to alleviate GAG storage in the MPS brain, a finding that has been further supported by other animal model studies as well. Based on these studies, intrathecal enzyme therapy has also been undertaken in a small number of MPS IH patients (36). Although some modest improvements have been reported, the long-term clinical outcomes and safety of this approach remain to be determined. These early studies have also highlighted some of the difficulties with repeat administration of enzymes by the intrathecal route.

The CNS-directed studies have similarly highlighted the importance of appropriate biomarkers to follow the effectiveness of treatment. For example, analysis of GAG storage in cerebrospinal fluid has been suggested, as well as measurement of the levels of the heparin cofactor II–thrombin complex (36). It is also recognized that inflammation plays an important role in the CNS disease of MPS animals and patients, and inflammatory biomarkers may be measured in the cerebrospinal fluid as well. In addition, anti-inflammatory therapies (see below) might be considered in combination with enzyme delivery to achieve maximal therapeutic benefit. For the CNS in particular, noninvasive biomarkers are likely to play an important role in assessing the effectiveness of any new therapies because clinical benefits in cognitive function and other CNS parameters could take many years to become measurable.

Another outcome of ERT that has emerged from the early experiences in MPS patients is that although the systemically administered enzymes are generally useful in improving soft tissues in the skeletal system of these patients (ligaments, tendons, etc.), they are not effective in the cartilage and bones themselves. Over time, therefore, the soft tissues cannot support the heavy, dense bones in these individuals, leading to additional bone complications, particularly in the spine. An unexpected outcome of ongoing ERT in these patients might therefore be a worsening of certain aspects of their bone disease—leading, for example, to more surgical intervention to correct spinal compressions (126).

This observation has led investigators to more carefully examine the mechanisms of cartilage and bone disease in MPS, with the goal of identifying additional therapies that could be used in combination with ERT to alleviate them. For example, it is now clear that GAG storage in MPS cartilage induces TLR4 signaling and TNF-α-mediated inflammation (103). Treatment of MPS animal models with anti-TNF-α antibody therapy significantly reduced articular chondrocyte death and improved both cartilage histology and growth plate organization (103). Synovial tissue hyperplasia characteristic of the MPS diseases was also reduced by anti-TNF-α therapy. Most important, when used in combination with ERT in a rat model of MPS VI, this therapy led to enhanced bone growth, increased motility, and markedly improved tracheal morphology (39). This proof-of-concept experiment demonstrated the importance of inflammation in MPS bone and joint disease and the value of anti-inflammatory combination therapies.

Finally, ERT experiences in MPS animal models and patients have also shown that very early intervention improves the effectiveness of ERT in the bones (and CNS, as mentioned above) (47, 112). These studies have highlighted the importance of newborn screening for these diseases and the importance of initiating therapy as soon as possible.

Pompe Disease

Pompe disease (glycogenosis type II) is an autosomal recessive disorder that results from the deficient activity of acid α-glucosidase and the lysosomal accumulation of glycogen, primarily

in smooth and skeletal muscle throughout the body. The infantile-onset form is characterized by hypertrophic cardiomyopathy, significant hypotonia, macroglossia, and death in the first year of life owing to cardiorespiratory failure. In contrast, the later-onset forms (childhood, juvenile, adult onset) present with progressive muscle weakness with involvement of the respiratory muscles. These patients can present as early as after the first year of life to as late as the sixth decade. With disease progression, patients can become wheelchair-bound and ventilator-dependent.

In Pompe disease, the challenge for ERT was to clear the accumulated glycogen from muscle (smooth and skeletal muscle); both types of tissue are hard to reach (**Table 3**), but the latter is more difficult, presumably owing to the low abundance of the mannose-6-phosphate receptor in skeletal muscle (73). Following preclinical studies in animal models (10, 11, 69), clinical trials were first conducted in infantile-onset patients with human recombinant acid α-glucosidase (alglucosidase alfa, Myozyme®, Genzyme Corporation) produced in CHO cells or in transgenic rabbits (1, 88, 114). Because muscle is hard to reach, enzyme doses of 20–40 mg kg^{-1} weekly or biweekly were needed to overcome the limited biodistribution to muscle cells, particularly the skeletal and respiratory system muscles (e.g., the diaphragm and external intercostal muscles); in the latter, these doses improved muscle morphology in both infantile- and later-onset patients.

ERT in infantile-onset patients has resulted in improved cardiac function and significantly decreased left ventricular wall thickness and mass. Clinical trials of acid α-glucosidase (20 mg kg^{-1} biweekly) in later-onset patients improved walking distance and stabilized neuromuscular and pulmonary function (107, 116). Overall, the response to ERT was generally positive, particularly with early treatment (71), and resulted in increased survival and improved motor function; however, the clinical response in patients has been remarkably variable.

The variable effectiveness of ERT in both subtypes is primarily due to several factors, including age/stage of disease at ERT start, muscle fiber type, defective autophagy, and immune response to the infused enzyme. The formation of antibodies is highly dependent on the patient's CRIM status (6), which in turn depends on the patient's specific acid α-glucosidase–encoding gene (*GAA*) mutations (3). CRIM-negative patients have no mutant enzyme protein and can raise high titers of IgG antibodies against the recombinant enzyme, thereby resulting in substrate reaccumulation and disease progression. CRIM-negative infantile-onset patients that develop high antibody titers have had a poor clinical response to ERT, with the disease continuing to progress to invasive ventilation or demise (88, 114). In contrast, most CRIM-positive infantile-onset patients have low antibody titers, develop tolerance, and improve with ERT. Importantly, a subset of such CRIM-positive patients also develop high antibody titers against the wild-type enzyme (likely due to the nature of the underlying mutations) and have an attenuated response to ERT (71).

To address these challenges, recent studies have attempted to predict the CRIM status of patients based on Western-blot analyses of cultured fibroblasts and the patients' *GAA* mutations (3). Of more than 240 patients studied, ~25% were CRIM negative; most of these patients had nonsense mutations, frameshift mutations, and/or large deletions. Initial genotyping and prediction of the CRIM status of newly identified patients are important for predicting the efficacy of ERT in Pompe disease, particularly because recent studies have shown that immunomodulation of CRIM-negative patients can lead to tolerization if initiated prior to or shortly after the initiation of ERT (38, 80, 108). Importantly, CRIM-negative patients have been successfully tolerized by a short course of immunomodulation with rituximab, methotrexate, and intravenous immunoglobulin (80). In those who did not tolerize after this regimen, a course of bortezomib did induce tolerance (5). Further experience is needed with immunomodulation

to induce tolerance in the setting of patients with high, sustained antibody titers.

Efforts are also under way to develop second-generation recombinant acid α-glucosidases (and other enzymes), either by increasing the mannose-6-phosphate content through neoglycosylation (131, 132) or by generating a chimeric fusion protein of acid α-glucosidase and IGF2, which efficiently binds the mannose-6-phosphate receptor (18, 51). A phase 1/2 open-label clinical trial is under way to evaluate IGF2 fusion enzyme at doses of 5, 10, and 20 mg kg^{-1} biweekly in later-onset patients (117).

Another intriguing approach under evaluation for Pompe disease that may also be applicable to other ERTs is the upregulation of the mannose-6-phosphate receptor gene (*MPR*) to enhance the number of receptors on cell surfaces for increased enzyme uptake. Studies in double-knockout mice with a muscle-specific conditional *MPR* knockout and a ubiquitous *GAA* knockout have shown how dependent enzyme uptake is on these receptors (73). Administration of the selective β(2) agonist (clenbuterol) enhanced *MPR* expression in skeletal muscle and other tissues, suggesting that the efficacy of ERT in Pompe disease and other LSDs may be enhanced by this combined therapy (73).

Moreover, the recent experience with newborn screening and early ERT resulted in markedly improved outcomes in Pompe disease (22). Therefore, newborn diagnosis, rapid prediction of the CRIM status by genotyping, and early initiation of ERT with immunomodulation in CRIM-negative patients may overcome some of the challenges in this and other LSDs and improve therapeutic outcomes.

Lysosomal Storage Diseases in Clinical Trials

Clinical trials are under way to develop ERT for four additional autosomal recessive LSDs: lysosomal acid lipase deficiency, type B Niemann-Pick disease, MPS IVA, and α-mannosidosis. On the basis of animal model studies, a phase 1/2 open-label trial has been completed in cholesteryl ester storage disease (CESD), the later-onset form of lysosomal acid lipase deficiency (45). CESD is characterized by progressive lysosomal accumulation of cholesterol esters and triglycerides, primarily in liver cells, leading to hepatosplenomegaly, fatty liver disease, cirrhosis, and liver failure. Affected patients also have type II hyperlipidemia and progressive vascular lipid deposition. Using a recombinant human enzyme made in an egg white expression system, investigators evaluated ERT at four weekly doses of 0.35, 1.0, or 3.0 mg kg^{-1} in adult CESD patients who subsequently enrolled in an extension trial. The drug was well tolerated and there were no infusion reactions (45).

A phase 1 open-label trial was also conducted in nonneuropathic type B Niemann-Pick disease (79), which is caused by a deficiency of acid sphingomyelinase and the accumulation of sphingomyelin. This subtype of the disease is characterized by hepatosplenomegaly, secondary hyperplenism, and pulmonary involvement. Patients received single enzyme doses ranging from 0.1 to 1.0 mg kg^{-1}. The drug was well tolerated at lower doses, but at doses of 0.6 and 1.0 mg kg^{-1} the cytokine and bilirubin levels were elevated, suggesting that future trials may implement a low-dose "debulking" strategy followed by progressive dose increases. This was the first LSD in which administration of a single dose caused any toxicity, which presumably resulted from the catabolism of the accumulated sphingomyelin to ceramide, a proapoptotic lipid.

MPS IVA is characterized by keratan sulfate accumulation leading to a severe systemic skeletal dysplasia, and normal intelligence. A phase 1/2 open-label safety and dose escalation study was conducted in patients who received the recombinant enzyme produced in CHO cells at doses of 0.1, 1.0, and 2.0 mg kg^{-1} weekly for three consecutive 13-week periods, followed by a 36–48-week continuation study at 1.0 mg kg^{-1} weekly (57). Subsequently, these patients were enrolled in an extension study at a dose of 2.0 mg kg^{-1} weekly. After two years

of ERT, urinary keratan sulfate had decreased and walk distance and stair climbing had generally improved. Based on the phase 1/2 results, the future phase 3 trial will be conducted at 2.0 mg kg^{-1} weekly.

A phase 1/2 clinical trial of ERT for α-mannosidosis is also currently under way (13). This disease is characterized by dysostosis multiplex, hearing loss, intellectual impairment, and recurrent infections. Patients are receiving 1.0 mg kg^{-1} of recombinant human α-mannosidase (Lamazym®, Zymenex) for 12 months. At 6 months, the urinary and cerebrospinal fluid oligosaccharides had decreased and motor function had improved. The enzyme was generally well tolerated; two patients developed IgE antibodies, but no anaphylaxis was observed.

The development of ERT in each of these disorders is encouraging, and pivotal phase 3 randomized, double-blind, placebo-controlled trials will be required to establish their safety and efficacy. ERT in MPS IVA and α-mannosidosis will be challenged by their significant bone and/or brain involvement.

COMBINATION THERAPY

In addition to ERT for LSDs, other therapeutic modalities are available, in clinical trials, or under development, either as monotherapies or in combination with ERT (56). As noted in the section on the mucopolysaccharidoses (above), HSCT has been undertaken in many of the MPS subtypes, and successful engraftment has proven effective in MPS I (58) and MPS VI (75). ERT has been performed prior to and following HSCT in MPS I, and the advantages have been recently discussed (28). Experiences in MPS animal models have also revealed the potential of combining ERT with targeted anti-inflammatory therapies, particularly for the skeletal system (39).

Oral substrate reduction therapies have also been designed to inhibit β-glucocerebrosidase synthase, thereby reducing glycosphingolipid synthesis and the rate of glycosphingolipid accumulation (for review, see 91). This approach has been applied to Gaucher disease. Miglustat (*N*-butyldeoxynojirimycin, Zavesca®, Actelion Pharmaceuticals) has been approved for the oral treatment of Gaucher disease based on clinical trials in type 1 and 3 patients (40, 99). As a monotherapy for neuronopathic type 3 patients, it did not significantly alter the neurodegenerative disease (99). A clinical trial of miglustat in combination with ERT for type 1 disease did not show significant benefits (40). More recently, oral eliglustat tartrate (Genz-112638, Genzyme Corporation) was evaluated in type 1 patients as a monotherapy (77). The oral treatment showed hematologic, visceral, and skeletal improvements. Notably, the long-term safety of these glycosphingolipid synthesis inhibitors remains unclear because, unlike ERTs, these small molecules are likely to alter the glycosphingolipid and ganglioside levels of multiple tissues throughout the body.

Another attractive approach for lysosomal and other genetic diseases resulting from enzyme misfolding and/or trafficking is pharmacologic chaperone therapy (PCT), which is the use of specific competitive, low-molecular-weight enzyme inhibitors to rescue misfolded or unstable mutant enzymes (34), thereby increasing their function. For most LSDs, certain mutations encode enzymes with residual enzymatic activity; typically, patients with these mutations have a milder attenuated phenotype than those whose mutations encode essentially no enzyme function or protein. Mutations that encode residual activity are excellent candidates for PCT. For example, in vitro and in vivo studies have demonstrated that the residual α-Gal A activity due to different mutations in affected males with later-onset Fabry disease could be enhanced by 1-deoxygalactonojirimycin (Amigal™, Amicus Therapeutics) (67). When this drug was coadministered with ERT in the Fabry murine model, the chaperone increased the stability of the recombinant enzyme in the circulation, increased its plasma half-life, and increased its uptake and substrate degradation in various tissues as compared with intravenously administered enzyme alone (9). Similar studies of

combined PCT and ERT have been reported in the murine model of Gaucher disease (68). Of note is that the hydrophobic, low-molecular-weight chaperones may cross the blood-brain barrier, diffuse through connective tissue matrices, and reach target sites of pathology that infused macromolecular lysosomal enzymes cannot.

A different approach to chaperone therapy for LSDs uses heat shock protein 70, which has been shown to stabilize lysosomes and reduce lysosomal pathology in cells from several different LSDs (70). It is thought that this heat shock protein achieves these effects by enhancing the interaction of the lysosomal enzyme acid sphingomyelinase with the lysosomal membrane lipid bismonophosphate, thereby stabilizing the lysosomal membranes. This approach has not been evaluated in LSD animal models.

In addition to the above therapies, efforts are under way to develop stop-codon read-through drugs to rescue truncation mutations (17) as well as gene and stem cell therapies (102). These strategies continue to be developed but have not matured sufficiently for pivotal clinical trials in the LSDs.

EARLY INTERVENTION AND NEWBORN SCREENING

Clinical trials and recent reports have emphasized the importance of early intervention in Fabry disease (4, 44), Pompe disease (22), and MPS I (47), II (112), and VI (46, 78). In MPS VI, studies of patients who were treated early compared with siblings treated at a later age have documented the remarkable improvement in the earlier-treated siblings (46, 78). Because untreated patients with infantile Pompe disease die in their first year of life, newborn screening was initiated in Taiwan to identify these patients and initiate ERT in the first weeks or months of life (21, 22). These results have been impressive, suggesting that early intervention for the treatable LSDs may avoid or significantly minimize disease manifestations, prevent irreversible pathology, and improve long-term outcomes. These results argue strongly for newborn screening for the treatable LSDs, coupled with confirmatory mutation analyses to identify the severe neurologic and later-onset forms so that appropriate counseling can be provided to parents. In addition, newborn screening will facilitate the identification of the affected newborns' older affected relatives (particularly in X-linked Fabry disease).

PRINCIPLES OF ENZYME REPLACEMENT THERAPY AND REMAINING CHALLENGES

As highlighted above, 20 years of experience in treating six LSDs have revealed the essential principles for ERT and identified the remaining challenges. The essential principles are as follows (see also sidebar, Principles for Effective Enzyme Replacement Therapy in Lysosomal Storage Disorders):

1. **Lysosomal enzyme biodistribution and tissue delivery are receptor-mediated.** Intravenously administered recombinant lysosomal enzymes are rapidly cleared from the circulation, primarily by the mannose-6-phosphate (mannose in Gaucher disease) receptor–mediated pathway for cellular uptake and lysosomal delivery. Thus, the administered recombinant enzymes must have their full complement of mannose-6-phosphate residues and be fully sialylated for maximal lysosomal delivery to organs other than the liver in which the Kupffer cells and hepatocytes will compete for and rapidly take up mannose-terminated and galactose-terminated glycoproteins, respectively, the latter via the asialofetuin receptor (106).
2. **Dose is critical.** The higher the dose, the greater the biodistribution, especially to cell types or sites that have limited uptake, like the heart and kidney. The recent demonstration of neural uptake and neuronal substrate clearance in MPS VII adult mice through high-dose enzyme administration clearly emphasizes this principle (121).

3. **Substrate clearance is dose-dependent.** Adequate doses are required to reduce substrate levels in certain organs where the enzyme biodistribution is low. For example, in type 1 Gaucher disease, the accumulated substrate in the liver and spleen is easily reached because these organs take up a significant proportion of the intravenously infused enzyme. However, the biodistribution and uptake by bones are markedly lower, and early ERT with realistic doses (1.6 mg kg^{-1} biweekly) is required to prevent or minimize the bone disease. Analogously, in the Fabry disease mouse model, the kidney and heart receive ~1% and ~0.1% of the infused dose, respectively (63). Thus, adequate doses (~1 mg kg^{-1} biweekly) are required for delivery to these organs in humans (41, 84). In the MPSs, the liver and spleen are easily reachable, but adequate doses must be given to reach the heart, cartilage, and bones (53, 66, 85). In Pompe disease, delivery to the heart and skeletal muscles requires very high doses (20–40 mg kg^{-1} weekly or biweekly) (1, 72).
4. **ERT requires continuous treatment for optimal outcomes.** Substrate reaccumulation occurs if ERT is interrupted or stopped. This may be related in part to cell turnover or to the proclivity of enlarged lysosomes. Although not well understood, substrate reaccumulation and clinical exacerbation do occur when therapy is stopped (8).
5. **ERT has proven safe and well tolerated.** Although infusion-associated reactions occur, presumably when patients develop IgG antibodies against the infused recombinant enzyme, these reactions are generally transient, can be managed conservatively, and are more frequent in patients with little or no residual enzyme activity. With time, patients may reduce their antibody titers and become tolerized to these enzymes. CRIM-negative and even CRIM-positive patients may raise high titers of antienzyme antibodies in Pompe disease and MPS II, and may require immunomodulation to overcome the antibody effects.

PRINCIPLES FOR EFFECTIVE ENZYME REPLACEMENT THERAPY IN LYSOSOMAL STORAGE DISEASES

Among the key principles revealed by 20 years of experience in ERT for LSDs are the following:

- Enzyme biodistribution and lysosomal delivery are receptor-mediated. Enzyme uptake is dependent on the receptor density on cell membranes (mannose-6-phosphate receptors for most LSDs, mannose and Limp2 receptors for Gaucher disease). Therefore, the enzymes' mannose-6-phosphate content and sialylation must be maximized for optimal lysosomal uptake for most LSDs. Macromolecular enzymes do not cross the blood-brain barrier.
- Enzyme delivery and substrate clearance are dose-dependent. Adequate doses are required for delivery to critical sites of pathology, which are disease-specific. Certain tissues are easy to reach; others are hard to reach and require higher doses (**Table 3**).
- Interruption or cessation of ERT leads to substrate reaccumulation and may exacerbate clinical manifestations.
- Immune reactions depend on the presence or absence of residual mutant enzyme proteins. CRIM status may be predicted by genotyping for some diseases, and initial/early immunomodulation may induce tolerance and optimize therapy.
- Early treatment improves clinical outcomes and may prevent irreversible disease. Newborn screening and early intervention offer optimal outcomes.

The remaining challenges are as follows:

1. **Delivery to difficult sites of pathology.** New techniques are needed to reach difficult sites of pathology, such as the bones, cartilage, and brain. These may include direct delivery of the enzymes (such as intrathecal administration for the brain, which is being evaluated for MPS) as well as intra-articular administration for the bones (which has been studied

in the MPS animal models). Several laboratories are also developing methods to increase the mannose-6-phosphate content of enzymes or create active and stable enzyme fusion proteins with cell-type-specific targeting sequences. New enzyme formulations are also being developed as an alternative or supplement to intravenous administration (e.g., aerosols for the lung and intramuscular injections for the muscles).

2. **Management of immunologic reactions to ERT.** In some patients and diseases, immunologic reactions to the infused enzymes may limit the efficacy of treatment. New protocols for early immunomodulation therefore need to be evaluated to determine their safety and long-term effectiveness in tolerizing individuals in order to continue and optimize therapy.
3. **Identification of appropriate biomarkers that reflect therapeutic effectiveness.** For some organs, clinical response may take many months or years to be recognized. Therefore, the identification of appropriate biomarkers that enable reliable prediction and/or monitoring of clinical responses is needed. Proteomics and metabolomics are likely to play important roles in this area, as is the availability of animal models that can be used to identify and evaluate the relevance of prospective biomarkers.
4. **Evaluation of combinational therapies.** It is clear that ERT will not be completely effective for all organs. Therefore, new combinational approaches need to be evaluated using drugs that enhance delivery to hard-to-reach tissues as well as drugs that target alternative and secondary pathological pathways, such as inflammation.
5. **Early identification of patients for early therapy.** Early intervention in animal models and patients results in markedly improved clinical responses. However, for most LSDs, early identification of patients remains a major challenge, particularly prior to the onset of irreversible organ damage. Newborn screening programs are being implemented that may overcome this obstacle to early identifications. However, in combination with these screening efforts, DNA-based and other methods should be developed to predict the disease subtypes and the likely occurrence of therapeutic response.
6. **Reduction in cost and accessibility of therapy.** As newborn screening programs are implemented and more patients are identified preclinically, the questions of when to implement therapy for each LSD and how to provide accessibility and reimbursement for therapy will have to be answered. These are likely to be important challenges that will need to be addressed in the upcoming decade.

SUMMARY

Although the past 20 years of experience have provided many important lessons and insights, ERT for the LSDs remains a highly active area of research, as new strategies are being developed to improve current therapies and to expand the number of diseases that can be effectively treated. In the future, second-generation enzymes and/or combination therapies may increase the clinical benefit for LSD patients. It is also likely that newborn screening will lead to early intervention and perhaps preclude the development of irreversible damage, and may even prevent certain manifestations. The future development of effective gene therapy and/or the early transplantation of gene-corrected stem cells for individual patients may prove therapeutic, or even curative for certain LSDs. Treatment of the neuropathic LSDs remains the greatest challenge, and it is likely that future genome testing will increasingly identify couples at risk for having children with these devastating conditions, permitting them to avoid these debilitating diseases by prenatal or preimplantation diagnoses. Suffice it to say

that numerous investigations are under way to develop new treatments and cures for the LSDs, and this should remain an exciting area of research for years to come.

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