# Lysosomal Storage Diseases: From Pathophysiology to Therapy

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## Abstract

Lysosomal storage diseases are a group of rare, inborn, metabolic errors characterized by deficiencies in normal lysosomal function and by intralysosomal accumulation of undegraded substrates. The past 25 years have been characterized by remarkable progress in the treatment of these diseases and by the development of multiple therapeutic approaches. These approaches include strategies aimed at increasing the residual activity of a missing enzyme (enzyme replacement therapy, hematopoietic stem cell transplantation, pharmacological chaperone therapy and gene therapy) and approaches based on reducing the flux of substrates to lysosomes. As knowledge has improved about the pathophysiology of lysosomal storage diseases, novel targets for therapy have been identified, and innovative treatment approaches are being developed.

## THE BIOLOGY OF LYSOSOMES

Lysosomes are catabolic organelles that break down and recycle a range of complex substrates, including glycosaminoglycans, sphingolipids, glycogen, and proteins. The catabolic function is performed through the concerted action of approximately 60 different acidic hydrolases that belong to different protein families, such as glycosidases, sulfatases, peptidases, phosphatases, lipases, and nucleases. Several lysosomal and nonlysosomal proteins participate in lysosomal functioning (e.g., by modulating the activity of lysosome-resident proteins).

Lysosomal catabolic function depends on direct interaction between hydrolases and substrates in the lysosomal lumen. Lysosomal enzymes are synthesized in the endoplasmic reticulum, where they adopt their native conformations and translocate to lysosomes via specialized pathways. The majority of these hydrolases are targeted to lysosomes through the addition of mannose-6-phosphate (M6P) residues onto the oligosaccharide moieties of the enzymes, a modification that takes place in the late Golgi compartment, and through the M6P receptor (MPR) pathway (1). Some specific enzymes do not depend on MPR for lysosomal delivery, such as β-glucocerebrosidase, which is transported to lysosomes by lysosomal integral membrane protein 2 (LIMP-2) (2).

Substrates are transported to lysosomes through different routes. Specialized endocytic mechanisms (phagocytosis, macropinocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, and clathrin- and caveolin-independent endocytosis) are preferentially exploited according to the nature of the cargo. Intracellular materials are transported to the lysosomal compartment mainly through autophagy, a process by which cells capture and convey their own cytoplasmic components and organelles to lysosomal degradation and recycling (3).

Recent studies have expanded our perspective on lysosomal function. They have shown that lysosomes are not only catabolic organelles, but also involved in fundamental cellular functions, including nutrient sensing, signaling, vesicle trafficking, and cellular growth, to name a few.

It has become evident that the lysosome plays an important part in nutrient sensing and in signaling pathways involved in cell metabolism and growth. The recent discovery that the mammalian target of rapamycin complex 1 (mTORC1) kinase complex, the master controller of cell growth, exerts its function on the lysosomal surface suggests that cell growth and cell catabolism are coregulated (4, 5). Recent studies have shown that the level of amino acids in the lysosomal lumen controls mTORC1 localization on the lysosomal surface. During starvation, mTORC1 inhibition potently activates autophagy.

Lysosomal function requires the concerted action of hydrolases, acidification machinery, and membrane proteins. It has been recently discovered that genes involved in lysosomal function belong to a gene network—the coordinated lysosomal expression and regulation (CLEAR) network—and are transcriptionally regulated by the lysosomal master gene *TFEB* (6). TFEB (transcription factor EB) positively regulates the expression of lysosomal genes, controls the number of lysosomes, and promotes degradation of lysosomal substrates.

TFEB-mediated regulation allows lysosomal function to adapt to different physiological and pathological conditions (e.g., starvation or storage). Interestingly, phosphorylation of TFEB by mTORC1 negatively regulates its activity by retaining it in the cytoplasm, thus blocking its nuclear translocation. During starvation, mTORC1 inhibition allows TFEB to translocate to the nucleus and perform its transcriptional activity. This is the first example of a lysosome-to-nucleus signaling mechanism (7).

## LYSOSOMAL STORAGE DISEASES

Lysosomal storage diseases (LSDs) are a group of more than 50 inherited metabolic disorders characterized by the intralysosomal accumulation of undegraded substrates. LSDs are traditionally

classified according to the chemical properties of the accumulated substrate. Individually, each of these disorders is rare. However, their cumulative prevalence is relatively high when compared with other groups of rare diseases, and prevalence is estimated to be approximately 1 in 8,000 live births (8).

The clinical consequence of substrate storage in multiple organs and systems is the variable association of visceral, ocular, hematological, skeletal, and neurological manifestations, and there is partial phenotypic overlap among different disorders. Symptoms may emerge at variable ages, in some cases starting in utero or during the newborn period (such as fetal hydrops), or becoming evident in late adulthood. In general, the diseases progress and evolve over time.

In some cases the manifestations of LSDs are highly peculiar and thus allow for a gestalt diagnosis. One of these peculiar features is the facial dysmorphism that gives patients a typical Hurler-like or gargoyle-like appearance. Visceral manifestations (hepatosplenomegaly) and hematological abnormalities (enlarged, substrate-filled vacuoles visible in lymphocytes or histiocytes) are also typical of LSDs. Skeletal involvement is characterized by generalized bone dysplasia, commonly referred to as dysostosis multiplex; joint limitations; abnormalities of bone density; areas of bone infarction; and osteonecrosis. Ocular manifestations include a range of abnormalities, such as corneal or lenticular opacities, retinal involvement (cherry red spot, retinal dystrophy), optic nerve atrophy, glaucoma, and blindness. About two-thirds of patients with LSDs display a significant neurological component, which is extremely variable and ranges from progressive neurodegeneration and severe cognitive impairment to epileptic, behavioral, and psychiatric disorders.

However, any other organ or system may be affected in LSDs, including the heart (cardiomegaly, valvular thickening, arrhythmias, and cardiac failure), skin (angiokeratoma, hypohidrosis), kidneys, upper respiratory tract, lungs, and intestine.

LSDs are often responsible for physical and neurological disabilities, and they have impacts on patients' health and life expectancy. For all these reasons, caring for patients with LSDs requires a multidisciplinary approach. Although remarkable progress in treatment has been made in recent years, these disorders remain associated with important unmet medical needs and heavy burdens in terms of public health and economic costs.

LSDs are caused by mutations in genes encoding soluble acidic hydrolases, integral membrane proteins, activator proteins, transporter proteins, or nonlysosomal proteins that are necessary for lysosomal function. Functional deficiencies in these proteins trigger a pathogenetic cascade that leads to intralysosomal accumulation of undegraded substrates in multiple tissues and organs (**Figure 1**).

Secondary impairment of other lysosome-related pathways may contribute to the pathology of LSDs. A prominent pathological feature of Pompe disease, a progressive myopathy caused by acid α-glucosidase (GAA) deficiency, is an expansion of the autophagic compartment in muscles (9, 10). In multiple sulfatase deficiency (MSD), which is caused by defective posttranslational activation of sulfatase-modifying factor 1 (SUMF-1) and simultaneous deficiency of all sulfatases, aberrant autophagy results from impaired fusion between autophagosomes and lysosomes (11). In Niemann–Pick disease type C1, a neurodegenerative disorder caused by mutations in the *NPC1* gene, sphingosine storage in lysosomes causes calcium depletion in these organelles, which results in secondary storage of cholesterol, sphingomyelin and glycosphingolipid (12). The abnormal composition of lysosomal membranes affects vesicle fusion and trafficking in MSD and in mucopolysaccharidosis (MPS) type IIIA, which is caused by heparan sulfamidase deficiency (13).

Perturbation of lysosomal function may also lead to less obvious consequences. A link has now been well established between impairment of lysosomal–autophagic pathways and Parkinson disease, which is the most prevalent neurodegenerative disorder; histopathologically, Parkinson disease is characterized by the accumulation of insoluble aggregates of the presynaptic protein

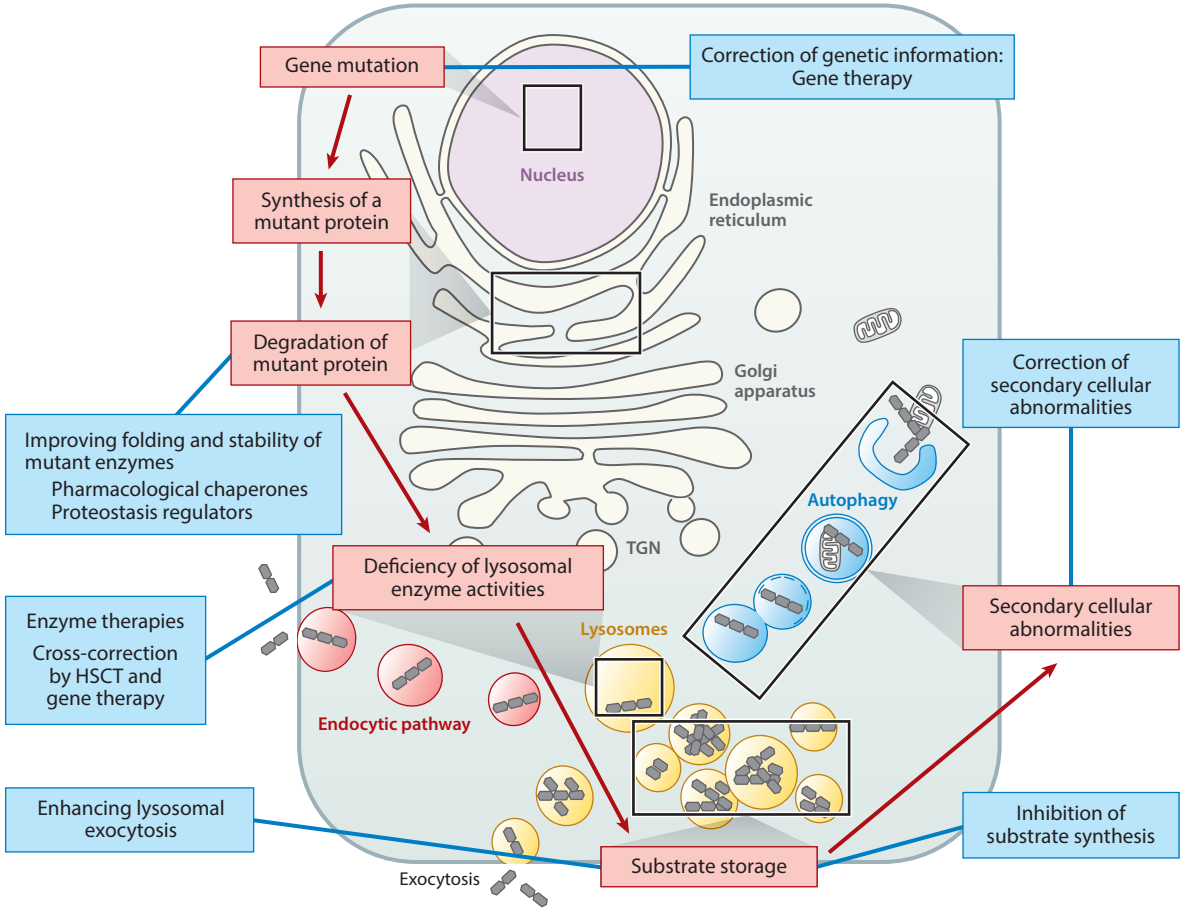


**Figure 1**

The pathogenetic cascade of lysosomal storage diseases and the therapeutic approaches to treating these disorders. Lysosomal storage diseases are caused by mutations in genes encoding proteins involved in the lysosomal functions. Missense mutations may cause degradation and retention in the endoplasmic reticulum, abnormal glycosylation, and mistrafficking of the mutant protein through the Golgi apparatus, trans-Golgi network (TGN), and to the lysosomes. Deficiency of lysosomal enzymes causes intra-lysosomal accumulation of undegraded substrates and secondary abnormalities of cellular pathways. Approaches developed to treat lysosomal storage diseases are based on different strategies, each targeted to a specific event in the pathogenetic cascade. The gene mutation may be corrected by delivering a wild-type copy of the mutated gene, which can direct the synthesis of the normal enzyme in the patient's cells. The mutant enzyme may be stabilized and protected from degradation, thus increasing its residual activity, with pharmacological chaperones or proteostasis regulators. The normal enzyme may be provided as a recombinant protein through the endocytic pathway of the patients' cells by using intravenous administration (enzyme replacement therapy, ERT). Alternatively, the normal enzyme can be provided as a precursor or secreted into the circulation by engineered patient cells or by allograft-transplanted cells (hematopoietic stem cell transplantation; HSCT). Other strategies are directed toward reducing substrate synthesis by enhancing the clearance of substrates from cells and tissues, or by manipulating specific cellular pathways (such as those involved in vesicle trafficking).

α-synuclein in typical intraneuronal inclusions (Lewy bodies) by the selective loss of dopaminergic neurons in the substantia nigra, and clinically by movement and postural defects. Although the mechanisms underlying this connection have not been fully elucidated, dysfunctions in several lysosomal proteins (and lysosomal gene mutations) have been implicated in the pathogenesis of Parkinson disease, with either loss-of-function or gain-of-function mechanisms. Examples of the

dysfunctional proteins (or mutated genes) implicated in Parkinson disease are β-glucocerebrosidase (14, 15) and lysosomal type 5 P-type ATPase (ATP13A2) (16, 17).

Mutations in genes encoding essential components of the endolysosomal–autophagic pathway have also been described in other neurodegenerative diseases, including Alzheimer disease, Huntington disease, frontotemporal dementia, and Charcot-Marie-Tooth type 2B (18).

## THE EVOLUTION IN PATIENT CARE

Until about two decades ago, the treatment of LSDs was exclusively based on multidisciplinary palliative therapy, and this approach remains a cornerstone in the care of LSD patients. Support therapies include managing neurological complications (such as neurosurgical decompression of the cervical spine and treatment of hydrocephalus, the use of anticonvulsant medication, and assistance for patients with learning disability); providing noninvasive or invasive ventilatory support; managing the increased risks of anesthesia; offering orthopedic interventions to alleviate spinal deformities, joint limitations, and retractions; providing nutritional support; treating cardiac involvement; and several others.

The past 25 years have been characterized by intensive and continual efforts to develop therapies specifically aimed at correcting the metabolic defects of these disorders. The approaches developed to treat LSDs are based on different strategies, each targeting a specific event in the pathogenetic cascade (**Figure 1**).

Most of these approaches are directed toward increasing the activity of the defective enzyme or protein. This can be achieved in different ways. The normal enzyme may be provided to the patient's cells through the endocytic pathway, by intravenous administration, as a recombinant protein. Alternatively, the normal enzyme can be provided as a precursor secreted into the circulation by engineered cells from the patient or by an allograft of transplanted cells. The gene mutation may be corrected by delivering a wild-type copy of the mutated gene that will direct the synthesis of the normal enzyme in the patient's cells. The mutant enzyme may be stabilized and protected from degradation, thus increasing its residual activity.

Other strategies are directed towards restoring the equilibrium between the synthesis of substrates and their degradation by lysosomal enzymes (the so-called storage equation of LSDs). This can be achieved by reducing substrate synthesis, by enhancing the clearance of substrates from cells and tissues, or by manipulating specific cellular pathways, such as those involved in vesicle trafficking.

In this review we focus on some of the therapies that are currently approved and in clinical use or that appear to hold promise for future advancements in the treatment of LSDs.

### Enzyme Replacement Therapy

Enzyme replacement therapy (ERT), considered the standard of care for several LSDs, is an excellent example of how progress in the field of lysosomal biology prompted the development of novel therapeutic approaches. The logic behind ERT evolved from pivotal studies on the mechanisms implicated in the sorting of newly synthesized lysosomal enzymes by way of the M6P and MPR pathways (19). Since the first studies in the early 1990s demonstrated the efficacy of ERT in Gaucher disease (20, 21), this approach has been extended to several other LSDs, including Fabry disease (22, 23), Pompe disease (24), and MPS I (25), II (26), and VI (27). Novel recombinant enzymes are currently being tested for the treatment of MPS IVA, MPS VII, MPS IIIA, metachromatic leukodystrophy, and acid lipase deficiency (**Table 1**). Comprehensive reviews of ERT are available (28, 29).

**Table 1 Approved treatments and selected clinical trials for lysosomal storage diseases**[a]

| Disease | Approved treatment; clinical trial<br>type of treatment, route of administration: therapeutic agent (company, sponsor) |
|---|---|
| Fabry disease | **Approved treatment**<br>**ERT, IV:** Agalsidase alfa (Shire)[b]; Agalsidase beta (Genzyme)<br>**Clinical trial**<br>**ERT, IV:** PRX-102 (Protalix Biotherapeutics);<br>**SRT, O:** Genz682452 (Genzyme, a Sanofi Company);<br>**ChT, O:** Migalastat HCl (Amicus Therapeutics);<br>**ChT, O** and **ERT, IV**: Migalastat HCl and either Agalsidase alfa or Agalsidase beta (Amicus Therapeutics);<br>**GT, IV:** RV-*GLA* (National Institute of Neurological Disorders and Stroke, United States) |
| Gaucher disease type I | **Approved treatment**<br>**ERT, IV:** Imiglucerase (Genzyme); Velaglucerase alfa (Shire); Taliglucerase alfa (Protalix BioTherapeutics)[c]<br>**SRT, O:** Miglustat (Actelion Pharmaceuticals); Eliglustat Tartrate (Genzyme Corp)[c]<br>**Clinical trial**<br>**ChT, O:** afegostat tartrate (Amicus Therapeutics); Ambroxol (Exsar Corporation);<br>**GT, IV:** RV-*GBA* (National Institute of Neurological Disorders and Stroke, United States) |
| GM1 and GM2 gangliosidoses | **Clinical trial**<br>**SRT, O:** Miglustat and ketogenic diet (University of Minnesota - Clinical and Translational Science Institute, United States) |
| GM2 gangliosidosis | **Clinical trial**<br>**ChT, O:** Pyrimethamine (The Hospital for Sick Children, Canada) |
| Lysosomal acid lipase deficiency | **Clinical trial**<br>**ERT, IV:** Sebelipase alfa (Synageva BioPharma) |
| Alpha-mannosidosis | **Clinical trial**<br>**ERT, IV:** Lamazym (Zymenex A/S) |
| Metachromatic leukodystrophy (late infantile) | **Clinical trial**<br>**ERT, IV:** Metazym (Shire)<br>**ERT, IT:** HGT-1110 (Shire)<br>**GT, IV:** LV-*ARSA* in CD34+ cells (IRCCS Ospedale San Raffaele/Fondazione Telethon, Italy)<br>**GT, IC:** AAVrh.10-*ARSA* (Institut National de la Santé et de la Recherche Médicale, France) |
| Mucopolysaccharidosis I | **Approved treatment**<br>**ERT, IV:** Laronidase (Genzyme)<br>**Clinical trial**<br>**ERT, IT:** Laronidase (Los Angeles Biomedical Research Institute, United States) |
| Mucopolysaccharidosis II | **Approved treatment**<br>**ERT, IV:** Idursulfase (Shire)<br>**Clinical trial**<br>**ERT, IV:** Idursulfase beta (Green Cross Corporation, Republic of Korea)<br>**ERT, IV and IT:** Idursulfase (Shire)<br>**GT, IV:** RV-*IDS* (National Institute of Child Health and Human Development, United States) |
| Mucopolysaccharidosis IIIA | **Clinical trial**<br>**ERT, IT:** HGT 1410 (Shire)<br>**GT, IC:** AAVrh.10-*SGHS* and *SUMF1* **(**Lysogene) |
| Mucopolysaccharidosis IIIB | **Clinical trial**<br>**GT, IC:** AAV5- *NAGLU* (Institut Pasteur, France) |

(*Continued*)

**Table 1** (*Continued*)

| Disease | Approved treatment; clinical trial<br>type of treatment, route of administration: therapeutic agent (company, sponsor) |
|---|---|
| Mucopolysaccharidoses III A, B, C | **Clinical trial**<br>**SRT, O:** Genistein aglycone (Central Manchester University Hospitals, United Kingdom) |
| Mucopolysaccharidosis IVA | **Approved treatment**<br>**ERT, IV:** Elosulfase alfa (BioMarin Pharmaceutical) |
| Mucopolysaccharidosis VI | **Approved treatment**<br>**ERT, IV:** Galsulfase (BioMarin Pharmaceutical) |
| Mucopolysaccharidosis VII | **Clinical trial**<br>**ERT, IV:** recombinant human beta-glucuronidase (Ultragenyx Pharmaceutical) |
| Neuronal ceroid lipofuscinosis, late infantile (CLN2) | **Clinical trial**<br>**GT, IC:** AAVrh.10-*CLN2* (Weill Medical College, Cornell University, United States)<br>**ERT, IC:** BMN 190 (BioMarin Pharmaceutical) |
| Niemann-Pick disease type B | **Clinical trial**<br>**ERT, IV:** recombinant human acid sphingomyelinase (Genzyme, a Sanofi Company) |
| Niemann-Pick disease type C | **Approved treatment**<br>**SRT, O:** Miglustat (Actelion Pharmaceuticals)[b]<br>**Clinical trial**<br>**Other, IC:** 2-hydroxypropyl-beta-cyclodextrin (National Institute of Child Health and Human Development, United States)<br>**Other, O:** Vorinostat (National Institute of Child Health and Human Development, United States) |
| Pompe disease | **Approved treatment**<br>**ERT, IV:** Alglucosidase alfa (Genzyme)<br>**Clinical trial**<br>**ERT, IV:** neoGAA (Genzyme, a Sanofi Company)<br>**ERT, IV:** GILT-tagged recombinant human (BioMarin Pharmaceutical)<br>**ERT, IV:** Alglucosidase alfa and albuterol (Duke University, United States)<br>**ERT, IV:** Alglucosidase alfa and clenbuterol (Duke University, United States)<br>**ChT, O:** duvoglustat HCl (Amicus Therapeutics)<br>**ERT, IV** and **ChT, O:** Alglucosidase alfa and miglustat (Università degli Studi di Napoli Federico II, Italy)<br>**ERT, IV** and **ChT, O:** Alglucosidase alfa and duvoglustat HCl (Amicus Therapeutics)<br>**GT, ID:** AAV1-*GAA* (University of Florida, United States) |

Abbreviations: AAV, adeno-associated viral vector; *ARSA*, arylsulfatase A gene; ChT, chaperone therapy; *CLN2*, neuronal ceroid lipofuscinosis 2 gene; ERT, enzyme replacement therapy; GILT, glycosylation-independent lysosomal targeting; *GAA*, alpha-glucosidase gene; *GBA*, beta-glucocerebrosidase gene; *GLA*,alpha-galactosidase A gene; GT, gene therapy; IC, intracerebral; ID, intradiaphragmatic; *IDS*, iduronate-2-sulfatase gene; IT, intrathecal; IV, intravenous; LV lentiviral vector; *NAGLU*, N-acetylglucosaminidase gene; O, oral; RV, retroviral vector; *SGHS*, N-sulfoglucosamine sulfohydrolase gene; SRT, substrate reduction therapy; *SUMF-1*, sulfatase-modifying factor 1 gene.

[a]Further information on approved treatments and clinical trials can be found on the Web. Useful addresses for registered clinical trials in the United States and Europe are: **https://www.clinicaltrials.gov/**; **https://eudract.ema.europa.eu/**.

[b]Approved by the European Medicines Agency but not by the US Food and Drug Administration.

[c]Approved by the US Food and Drug Administration but not by the European Medicines Agency.

Now, after the first phase of ERT development, there are sufficient clinical data to document the successes of this approach. However, important limitations have emerged, and it is necessary to develop strategies to improve its efficacy.

Significant variability in clinical benefit has been observed among different patients. After an initial period of improvement, the disease may again progress in some patients. In MPS I, II,

and VI, the correction of pathology is insufficient in tissues such as bone, cartilage, and heart (30), which are major pathological sites. In Pompe disease, skeletal muscle pathology remains partly refractory to ERT, particularly in patients with advanced stages of the disease (31, 32). The benefits and cost effectiveness of ERT have been carefully evaluated in a multicenter study in the United Kingdom (33).

A major drawback of ERT has been the limited bioavailability of intravenously injected recombinant enzymes. Recombinant enzymes are large molecules that do not freely diffuse across membranes and are unable to reach therapeutic concentrations in some target tissues, particularly the brain. A major challenge in the coming years will be to discover a means that allows recombinant enzymes to cross the blood–brain barrier (BBB). Given that approximately two-thirds of LSDs cause neurological symptoms and progressive neurodegeneration, the major therapeutic goals in treating many LSDs are to obtain corrective enzyme levels in the brain and correct the pathology in the central nervous system (34, 35).

Strategies to improve the delivery of enzymes to the central nervous system are currently undergoing preclinical and clinical evaluation. For example, β-glucuronidase, which is deficient in MPS VII, has been chemically modified to increase its plasma half-life and facilitate its traffic through the BBB (36, 37). Other approaches, based on the use of so-called Trojan horses, rely on chimeric enzymes fused with peptides to allow penetration of the BBB and brain delivery through specific pathways (such as the apolipoprotein and receptor pathways). These approaches have been evaluated in preclinical studies for α-iduronidase (38, 39), iduronate-2-sulfatase (40), arylsulfatase A (41), and tripeptidyl peptidase I (42). Sorrentino et al. (43) showed that a chimeric heparan sulfamidase, produced by liver cells after adeno-associated virus (AAV) 2/8 gene delivery, could cross the BBB via transcytosis and correct the enzyme deficiency in a mouse model of MPS IIIA. The chimeric enzyme was engineered by adding the signal peptide from the highly secreted iduronate-2-sulfatase and the BBB-binding domain of apolipoprotein B.

In addition to these approaches, preclinical studies are evaluating the use of invasive procedures to deliver recombinant enzymes directly into the cerebrospinal fluid as treatment for several lysosomal disorders. The objectives of these approaches are to alleviate spinal cord compression and to improve the neurological or cognitive outcome of patients. Intrathecal administration and lumbar or cisterna magna puncture have been studied in animal models of various types of mucopolysaccharidoses (44–47). Intrathecal ERT has been translated into human therapy for mucopolysaccharidoses types I and VI (48). Devices for continuous intrathecal infusion have been developed and tested in preclinical studies. Some clinical trials of intrathecal administration of ERT are ongoing, and others have been completed (**Table 1**).

Attempts have also been made to improve the efficiency of ERT targeting muscle in Pompe disease by enriching the recombinant enzyme with M6P moieties (49, 50) or by producing a chimeric enzyme fused with a peptide derived from insulin-like growth factor 2 (51, 52). Enhanced lysosomal transport of recombinant enzymes has also been obtained in animal models of Pompe and Fabry diseases by coupling the enzymes with polymer nanocarriers coated with an antibody that is specific to intercellular adhesion molecule-1 (ICAM-1) (53, 54).

An alternative strategy to improve muscle targeting by recombinant human GAA is based on the enhancing effect of β2-agonists on the expression of the MPR at the plasma membrane of myofibers (55, 56). The use of β-2-agonists as adjunctive therapy to ERT is being evaluated in a clinical trial (**Table 1**).

Another limitation of ERT is the patient's immune response to recombinant enzymes, mainly in patients who are cross-reactive material–negative (57). Increased antibody titers have been associated with severe immune reactions and a reduced clinical efficacy for ERT. Immune modulation has been used to prevent these problems in some LSDs, such as Pompe disease (58, 59).

Novel manufacturing procedures are being developed, and these are expected to lower the high costs of ERT. Treating a single LSD patient may cost as much as several hundred thousand dollars per year. Such an expense may limit patients' access to ERT and heavily impact the budgets of national health systems (33). Recombinant β-glucocerebrosidase has been manufactured in plants or plant-cell expression systems, and in 2012 it was approved for clinical use to treat Gaucher disease (60). Other plant-based production processes have been either reported in the literature or patented (61–63).

## Hematopoietic Stem Cell Transplantation

Hematopoietic stem cell transplantation uses hematopoietic stem cells derived from a healthy donor as a therapeutic agent. These cells have a dual effect: they can both repopulate specific tissues and secrete functional lysosomal hydrolases in the extracellular space and into blood circulation. The secreted normal enzyme can be recaptured by the recipient cells and cross-correct the enzyme defect in these cells. This approach is effective only for some LSDs; it has been shown to be ineffective for several others. The best results are obtained when the procedure is performed in a restricted therapeutic window, early in the course of the disease, and in specific subsets of patients. Thus, guidelines have been developed to determine which patients are eligible and to promote timely intervention (64, 65).

## Pharmacological Chaperone Therapy

Pharmacological chaperone therapy is based on the concept that loss-of-function diseases are often caused by missense mutations that disrupt the three-dimensional conformation of mutant proteins. Misfolded proteins may be recognized by the quality control systems of the endoplasmic reticulum (ER) and degraded, retained in the ER, or abnormally glycosylated and mistrafficked (66). Thus, in these protein misfolding diseases the loss of function is not due to the loss of catalytic activity but rather is the result of degradation or inappropriate trafficking of the aberrant protein. Small-molecule ligands (pharmacological chaperones) can interact with mutant proteins, favor their native conformation, enhance their stability, and allow for correct trafficking. As a result, the enzymatic activity of the mutant protein is partially rescued. Chaperone therapy has been proposed as a feasible strategy for treating some LSDs (66–68) and is presently under evaluation in phase I/II clinical trials (**Table 1**).

An important evolution in pharmacological chaperone therapy was the demonstration that some chaperones not only are able to rescue the endogenous, mutant, misfolded proteins, but can also enhance the physical stability, and possibly the efficacy, of the wild-type recombinant enzymes that are commonly used for ERT. This effect was demonstrated in preclinical in vitro and in vivo studies for Pompe, Fabry, and Gaucher diseases (69–73). Some trials evaluating the potential of the combination of ERT and chaperones are in progress, and others have already been completed (74) (**Table 1**).

## Proteostasis Regulators

Other small-molecule-based approaches have been proposed to rescue the mutated enzymes in LSDs. Chaperones are ligands that specifically interact with mutant proteins (and are thus able to rescue a single protein), but proteostasis regulators can adjust the capacity for proteostasis, a complex network that controls protein synthesis, folding, trafficking, aggregation, and degradation. Regulation of proteostasis has been proposed as a strategy to treat two distinct LSDs caused by a deficiency of β-hexosaminidase A, Gaucher disease and GM2 gangliosidosis (Tay–Sachs disease)

(75). Regulation of proteostasis has also been achieved through the overexpression of the *TFEB* gene in Gaucher disease fibroblasts (76). Downregulation of the ER-resident protein FKBP10 also resulted in enhanced proteostasis of β-glucocerebrosidase (77).

## Substrate Reduction Therapy

Substrate reduction therapy inhibits specific steps in the biosynthetic pathways of substrates to restore the equilibrium between the synthesis of substrates and their degradation by lysosomal enzymes (78, 79). This task is generally accomplished by using small-molecule enzyme inhibitors that are involved in substrate biosynthesis. A substrate-reducing agent, miglustat (Actelion Pharmaceuticals, San Francisco, CA), has already been approved for clinical use to treat type 1 Gaucher disease (80) and Niemann–Pick disease type C (81). A novel substrate inhibitor, eliglustat tartrate (Genzyme, a Sanofi Company, Cambridge, MA), has been introduced recently and evaluated in a phase II clinical trial (82) for treating Gaucher disease. The flavonoid genistein has been proposed as a treatment for MPS (83). A phase III clinical trial using high-dose oral genistein aglycone is in progress (**Table 1**).

## Gene Therapy

Gene therapy is directed toward increasing or restoring defective enzyme activity in patients' cells and tissues by delivering a wild-type copy of the defective gene. LSDs appear to be excellent candidates for gene therapy (84). These disorders are monogenic and, in principle, it is possible to cure the disease by correcting the gene defect. In addition, small increases in enzymatic activity (as little as <10%) may be sufficient to produce clinical benefit and phenotypic correction of LSDs.

Although this approach is generally based on delivering the therapeutic gene to patients using viral vectors as carriers, a broad range of different strategies may be exploited, depending on the tissues that need to be targeted and on the characteristics of the protein or enzyme that must be replaced.

A first approach is to correct the genetic information in all of the patient's cells and tissues by systemically delivering the therapeutic gene under the control of a ubiquitous promoter in order to allow synthesis of the wild-type protein in situ. However, because most lysosomal hydrolases are secreted and can be recaptured by neighboring or distant cells via the MPR pathway, an alternative strategy may be based on transducing specific cells or organs that may act as protein factories and thus cross-correct other tissues. This approach has advantages when compared with ERT because factory cells provide stable and sustained amounts of the normal enzyme in the blood circulation.

Transduction of specific tissues or cells can be achieved in various ways. An in vivo strategy may use systemic delivery and a tissue-specific promoter or direct injection into a target organ. Alternatively, an ex vivo strategy has also been developed that engineers a patient's bone marrow cells and then injects the engineered cells back into the patient.

Depending on the delivery strategy being used, different viral vectors and promoters that control the expression of the gene of interest can be used as therapeutic agents. Currently, AAVs are the vector of choice for in vivo gene therapy, and lentiviruses are the vectors of choice for ex vivo gene therapy.

Preclinical data have been obtained from small- and large-animal models of LSDs for all of these strategies and viral vectors. Some viral vectors have been designated as orphan drugs and are to be tested in clinical trials (**Table 1**). For some diseases—including MPS II, MPS IIIA, MPS VI, Pompe disease, neuronal ceroid lipofuscinoses, Gaucher disease, and Fabry disease—clinical trials are already in progress (**Table 1**). The ex vivo approach has been successfully used in a clinical

trial to treat patients affected by metachromatic leukodystrophy (MLD), a severe and progressive neurodegenerative LSD caused by arylsulfatase A (ARSA) deficiency. A lentivirus vector has been used to transfer a functional *ARSA* gene into hematopoietic stem cells from three patients with presymptomatic, late-infantile MLD (85). After reinfusion of the gene-corrected hematopoietic cells, all patients showed correction of enzyme levels and arrest of neurodegeneration, indicating the clinical efficacy of this approach.

## ADVANCES IN UNDERSTANDING PATHOPHYSIOLOGY AND ITS IMPACT ON THE DEVELOPMENT OF TREATMENTS

In recent years novel areas of therapeutic intervention have been identified in addition to those typically directed towards correcting the enzyme defect and reducing storage. Some of the examples below clearly show that as the lysosomal biology is revealed, new therapeutic strategies can be devised, in most cases based on the manipulation of specific molecular pathways.

Manipulation of the autophagic pathway has been reported as a potential target for therapy in Pompe disease (86). Genetic suppression of autophagy in the animal model of Pompe disease resulted in a substantial reduction in glycogen accumulation in skeletal muscle and in improved efficacy of ERT.

Stimulation of lysosomal exocytosis has also been identified as a strategy for treating LSDs. Lysosomes are able to secrete their contents through a $Ca^{2+}$-dependent process. Medina et al. (87) showed that this process is modulated by TFEB, and that overexpression and activation of TFEB result in enhanced clearance of substrates in cells in two LSDs: MSD and Pompe disease. Overexpression of TFEB reduced glycogen load and lysosomal size, improved autophagosome processing, and alleviated excessive accumulation of autophagic vacuoles in cultured myoblasts from a Pompe disease murine model (88). In vivo *TFEB* gene delivery mediated by AAV2/1 administered by intramuscular injection resulted in nearly complete glycogen clearance and restored muscle architecture. *TFEB*-induced cellular clearance of stored substrates through enhanced exocytosis appears to be particularly attractive because it may be obtained in all LSDs, irrespective of the metabolic defect.

Cyclodextrin, a cholesterol-sequestering agent, has been shown to mobilize cholesterol from late endosomes and lysosomes, bypassing the functions of the genes *NPC1* and *NPC2*, which are involved in Niemann–Pick disease type C. Clinical trials using cyclodextrin to treat this disorder are in progress (**Table 1**). In the same disorder, other studies have shown that depletion of lysosomal $Ca^{2+}$ stores is another potential target for therapeutic intervention and that curcumin could compensate for this calcium defect in lysosomes (12). The combined use of curcumin and the nonsteroidal anti-inflammatory drug ibuprofen led to improved neuroprotection in an *NPC1*-defective mouse model (89).

Heat-shock protein 70 (Hsp70) has been shown to enhance lysosomal stability by modulating the sphingolipid composition of membranes. In cells from patients with Niemann–Pick disease type A, acid sphingomyelinase deficiency could be partially restored by treatment with Hsp70, indicating that this may be an additional strategy for treating LSDs (90, 91).

## CONCLUSIONS

In spite of the remarkable progress that has been made in treating LSDs, these disorders still represent a large and growing unmet medical need. Future research must confront important challenges. Relatively short-term tasks are to translate into clinical use the strategies that are currently under development and to optimize existing therapies. In particular, it will be important

to improve the bioavailability and targeting of therapeutic agents, to reduce the impact of therapies on patients' quality of life, and to lower the costs of therapies.

Researchers also need to explore the efficacy of therapeutic protocols based on the combination of different approaches, to address all the manifestations of multisystem disorders such as LSDs, and to consider the development of personalized therapies. Finally, a major and exciting challenge will be to improve the understanding of the pathophysiology of LSDs to identify new targets for therapy.

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