Treatment options for lysosomal storage disorders: developing insights

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Introduction: Lysosomal storage disorders (LSDs) are clinically heterogeneous disorders that result primarily from lysosomal accumulation of macromolecules in various tissues. LSDs are always progressive, and often lead to severe symptoms and premature death. The identification of the underlying genetic and enzymatic defects has prompted the development of various treatment options.

Areas covered: To describe the current treatment options for LSDs, the authors provide a focused overview of their pathophysiology. They discuss the current applications and challenges of enzyme-replacement therapy, stem-cell therapy, gene therapy, chaperone therapy and substrate-reduction therapy, as well as future therapeutic prospects.

Expert opinion: Over recent decades, considerable progress has been made in the treatment of LSDs and in the outcome of patients. None of the current options are completely curative yet. They are complicated by the difficulty in efficiently targeting all affected tissues (particularly the central nervous system), in reaching sufficiently high enzyme levels in the target tissues, and by their high costs. The pathways leading from the genetic mutation to the clinical symptoms should be further elucidated, as they might prompt the development of new and ultimately curative therapies.

Keywords: bone marrow transplantation, chaperone therapy, enzyme replacement therapy, gene therapy, lysosomal storage disease, lysosomal storage disorder, stem cell transplantation, substrate reduction therapy

1. Introduction

1.1 General introduction
Most lysosomal storage disorders (LSDs) are severe, progressive disorders that affect various tissues, often leading to severe symptoms and premature death. Over recent decades, major progress has been made and several therapies have been developed. Without being completely curative, these improve the clinical outcome of patients with several LSDs.

This review discusses the mechanism and current applications of enzyme-replacement therapy (ERT), hematopoietic stem-cell therapy (HSCT), gene therapy, chaperone therapy and substrate-reduction therapy (SRT), examining their pros and cons and the possible directions of future treatment options. First, the authors discuss lysosomal function and the pathophysiology of LSDs.

1.2 Lysosomal function and lysosomal proteins
Lysosomes were first described in 1955 as membrane-bound cytoplasmic 'bodies' – that is, organelles – filled with acid hydrolases that are able to degrade a
Luminal side [3]. Among others, these proteins are thought
lysosomal-associated membrane protein (LAMP)-1 and
related lysosomal-associated membrane proteins:
2
and Danon disease are X-linked disorders; all others are autoso-
though the individual LSDs are rare, their combined
prevalence is estimated to be 1:7000 live births [14,15].
Figure 1A shows the pathophysiological cascade. Deficiency
in a lysosomal function leads primarily to the accumulation of
one or more undegradable substances within the lysosomes.
Lysosomal dysfunction occurs, with other cell systems becom-
ing involved – for example, reduced cell and tissue renewal
through defective endocytosis and autophagy. Alterations in
signal-transduction pathways and intracellular calcium
homeostasis are tertiary events [16,17]. The initial cellular
pathology causes tissue damage followed by organ dysfunc-
tion, and can lead to more distant effects, for instance,
through the formation and circulation of toxic substances or
through immune irregularities [18].
Due to the presence of lysosomes in all different cell
types except erythrocytes, the pathologic events are rarely
wide variety of macromolecular compounds, even including
entire mitochondria [1]. They are currently known to play var-
ious critical roles in cellular function and tissue homeostasis.
For instance, they are involved in pathogen defense (macro-
phages and neutrophilic granulocytes), bone remodeling (osteoclasts), cholesterol homeostasis and tissue-wide cell
renewal [2].
The lysosomal membrane is particularly rich in two struc-
turally related lysosomal-associated membrane proteins: lysosomal-associated membrane protein (LAMP)-1 and
LAMP-2, proteins that are highly glycosylated on the
luminal side [5]. Among others, these proteins are thought
to protect the membrane against degradation by its highly
aggressive content of an estimated 50 hydrolases, which
together allow the degradation of the many biological
substances that are brought to the lysosomes by three main
processes: phagocytosis, endocytosis and autophagy [4]. The
hydrolases include glycosidases, proteases, lipases and
DNA/RNAases. Other lysosomal membrane proteins func-
tion as a proton pump to acidify the lysosomal interior, or
as a transporter to export particular monosaccharides and
amino acids [3]. More functions of the lysosomal membrane
proteins will certainly be clarified, such as those of the
lysosomal integral membrane protein 2 (LIMP-2), which
was fairly recently discovered to be involved in the lysosomal
targeting of glucocerebrosidase [5].
Another class of lysosomal proteins is the activator proteins,
for example, saposins, which assist sphingolipid-degrading
enzymes by making the substrates accessible to the enzymes [6].
To fulfill their function, lysosomes constantly fuse with endo-
somes, autophagosomes, phagosomes and other membranous
structures including the cell membrane [4].
The hydrolytic lysosomal proteins themselves are macromo-
lecules that cannot pass the lysosomal membrane. They enter
the endoplasmic reticulum (ER) enacted by a signal peptide
at the extreme N-terminal end of the protein (Figure 1A). After

co-translational entry into the ER lumen, lysosomal proteins
become glycosylated, are folded and undergo a series of
post-translational modification events involving both the
carbohydrate side chains and the protein core. In this process,
most lysosomal enzymes acquire mannose 6-phosphate
(M6P) as a recognition marker for mannose 6-phosphate
receptor (M6PR)-mediated vesicular transport from trans-
Golgi to lysosomes [7]. Some lysosomal hydrolases reach
the lysosomes in a different way, such as glucocerebrosidase which
is directed to the lysosomes through binding to LIMP-2 in the
ER [5]. In physiological conditions, about 5 – 20% of the newly
synthesized lysosomal enzymes are secreted [7].
The lysosomal membrane proteins bear cytosolic sorting
signals that target them to the lysosomes through a
direct intracellular route or indirectly through the plasma
membrane [7].

1.3 Pathophysiology and clinical presentation
At present, up to 50 LSDs are known (refer to [8] for a detailed
description). Most are caused by mutations in genes coding
for one of the many hydrolases. These mutations lead to enzyme
deficiency or dysfunction and subsequent accumulation of
biological compounds. A minority of LSDs are caused by
mutations in lysosomal membrane transporter proteins (e.g.,
cystinosis and Salla disease) [9]. Interestingly, LAMP-2 defi-
ciency hampers the fusion of autophagosomes and lysosomes,
and is characterized by extralysosomal vesicular storage of glyco-
gen in the heart (Danon disease) [10]. LIMP-2 deficiency results
in action myoclonus-renal failure syndrome, which is clinically
similar to Gaucher type 3 disease [11], and may serve as a
modifier in Gaucher disease [12]. Mucolipidosis II (I-cell disease)
and mucolipidosis III are exceptional in that malfunction of
enzymes involved in the generation of the M6P recognition
marker leads to a plethora of lysosomal enzyme deficiencies [13].
Deficiencies of saposins, that is, sphingolipid activator proteins,
clinically mimic the sphingolipid storage disorders [6].
All LSDs are monogenic disorders and are inherited as recess-
itive traits. Mucopolysaccharidosis (MPS) type II, Fabry disease
and Danon disease are X-linked disorders; all others are auto-
somal. Though the individual LSDs are rare, their combined
prevalence is estimated to be 1:7000 live births [14,15].

This box summarizes key points contained in the article.
restricted to one cell type or tissue type, although there is usually one leading organ. Which organs are affected is largely determined by the cell- and organ-specific turnover of the storage compounds.

Most LSDs have a broad clinical spectrum, ranging from severe, rapidly progressing, infantile-onset forms to less progressive forms presenting in childhood or adulthood [8]. The differences in phenotype within a disease can be explained largely by the residual activity of the deficient enzyme, which results from different mutations within the same gene [19]. However, this genotype–phenotype correlation is not strict, and disease severity and progression can differ substantially, even within siblings or twins, indicating the involvement of other genetic or non-genetic modifying factors [20].

The pathophysiological mechanism of LSDs is complicated and still not fully understood. If it is further unraveled, new therapeutic targets may be identified [17].

1.4 Therapy
Several options for therapeutic intervention are provided by current insights not only into the role of lysosomes and their functioning through interaction with endocytic and autophagic systems, but also into the pathophysiology of lysosomal diseases. The objective of all these options is to relieve the burden of lysosomal storage and to restore cellular and organ functions by preventing storage or its recurrence. While certain therapeutic approaches apply overall, each disease offers unique challenges that are mainly related to the target tissues.

Figure 1B provides a schematic overview of the mechanisms of action of ERT, HSCT, gene therapy, chaperone therapy and SRT. These options are discussed in turn below. Table 1 provides an overview of the LSDs for which one or more of these treatments are presently available or evaluated in clinical trials.

2. Treatment options

2.1 Enzyme-replacement therapy

2.1.1 Principles
ERT is currently the commonest treatment for LSDs. The discovery of the first lysosomal enzyme defect in 1963 [21]
Table 1. Summary of the treatment options available or in trial for LSDs*.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Defective enzyme</th>
<th>Main clinical manifestations‡ [8]</th>
<th>Current treatment</th>
<th>Clinical trials (phase) [44]</th>
<th>OMIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucopolysaccharidoses</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>MPS type I</td>
<td>α-L-iduronidase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hemias, macroglossia</td>
<td>ERT, HSCT*</td>
<td>CSF-ERT* (I), HSCT** ERT§ (II), HSCT** CSF-ERT (I)</td>
<td>607014#, 607015#, 607016#</td>
</tr>
<tr>
<td>MPS type II</td>
<td>Iduronate 2-sulfatase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, hemias, macroglossia</td>
<td>ERT</td>
<td>CSF-ERT** ERT (V II), HSCT (II)</td>
<td>309900#</td>
</tr>
<tr>
<td>MPS type IIIA</td>
<td>N-Sulfoglucosamine sulfohydrolase</td>
<td>CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss</td>
<td>CSF-ERT (I/II), GT (1/2), HSCT (II)</td>
<td>HSCT (II)</td>
<td>252900#</td>
</tr>
<tr>
<td>MPS type IIIB</td>
<td>N-α-Acetylgalactosaminidase</td>
<td>CNS involvement with relatively mild somatic features, mild dysostosis multiplex, hepatosplenomegaly, hearing loss</td>
<td>HSCT (II)</td>
<td></td>
<td>252920#</td>
</tr>
<tr>
<td>MPS type IIIC</td>
<td>Heparan acetyl-CoA: α-glucosaminidase N-acetyltransferase</td>
<td>CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss</td>
<td></td>
<td></td>
<td>252930#</td>
</tr>
<tr>
<td>MPS type IIID</td>
<td>N-Acetylglucosamine-6-sulfatase</td>
<td>CNS involvement with relatively mild somatic disease, mild dysostosis multiplex, hepatosplenomegaly, hearing loss</td>
<td>HSCT (II)</td>
<td></td>
<td>252940#</td>
</tr>
<tr>
<td>MPS type IVA</td>
<td>Galactosamine-6-sulfate sulfatase</td>
<td>Dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatomegaly, corneal clouding</td>
<td>ERT (II)</td>
<td></td>
<td>253000#</td>
</tr>
<tr>
<td>MPS type VI</td>
<td>N-Acetylgalactosamine 4-sulfatase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hemias</td>
<td>ERT</td>
<td>HSCT (II)</td>
<td>253200#</td>
</tr>
<tr>
<td>MPS type VII</td>
<td>β-Glucuronidase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory problems, hearing loss, hepatosplenomegaly, corneal clouding, hemias</td>
<td>HSCT (II)</td>
<td></td>
<td>253220#</td>
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<tr>
<td>Oligosaccharidoses</td>
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<tr>
<td>Fucosidosis</td>
<td>α-L-fucosidase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hearing loss, hepatosplenomegaly, angiokeratoma</td>
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<td>230000#</td>
</tr>
<tr>
<td>α-Mannosidosis</td>
<td>α-Mannosidase</td>
<td>CNS involvement, dysostosis multiplex, ENT problems, hearing loss, hepatosplenomegaly, corneal clouding, hemias, immune deficiencies</td>
<td>ERT (II), HSCT (II)</td>
<td></td>
<td>248500#</td>
</tr>
</tbody>
</table>

The LSDs are listed according to the chemical properties of the accumulating substances.

*The LSDs for which no treatment is currently available or in clinical trial are not included.

‡As most LSDs present as a broad clinical spectrum, the main clinical manifestations per LSD are summarized, which may not be applicable for all patients individually.

§Only for Hurler patients.

**Only for Gaucher disease type I and III.

*Only for Gaucher disease type I.

**Only for mucolipidosis type II.

CNS: Central nervous system; CSF-ERT: Intracerebrospinal fluid enzyme-replacement therapy; CT: Chaperone therapy; ERT: Enzyme-replacement therapy; HSCT: Hematopoietic stem-cell transplantation; GT: Gene therapy; LSD: Lysosomal storage disorder; NCL: Neuronal ceroid lipofuscinosis; NPC: Niemann–Pick disease type C; PNS: Peripheral nervous system; SRT: Substrate-reduction therapy.
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<th>OMIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartylglucosaminuria</td>
<td>Aspartylglucosaminidase</td>
<td>CNS involvement, dysostosis multiplex, respiratory and ENT problems, hernias</td>
<td>HSCT (II)</td>
<td>208400#</td>
<td></td>
</tr>
<tr>
<td>Sphingolipidoses</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>GM1 gangliosidosis/Morquio B</td>
<td>β-Galactosidase</td>
<td>CNS involvement, dysostosis multiplex, hearing loss, hepatosplenomegaly, corneal clouding, loss of vision, macroglossia</td>
<td>HSCT (II)</td>
<td>230500#, 230600#, #230650#, 253010#</td>
<td></td>
</tr>
<tr>
<td>Sphingolipidoses</td>
<td></td>
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<tr>
<td>GM2 gangliosidosis(Tay-Sachs)</td>
<td>β-Hexosaminidase A</td>
<td>CNS involvement, ENT problems, hearing problems, loss of vision</td>
<td>CT (II), HSCT (II/III), SRT (II)</td>
<td>272900#</td>
<td></td>
</tr>
<tr>
<td>GM2 gangliosidosis(Sandhoff)</td>
<td>β-Hexosaminidase A and B</td>
<td>CNS involvement, ENT problems, hepatosplenomegaly, loss of vision</td>
<td>CT (II), HSCT (II/III), SRT (II)</td>
<td>268800#</td>
<td></td>
</tr>
<tr>
<td>Metachromatic leukodystrophy</td>
<td>Arylsulfatase A</td>
<td>CNS and PNS involvement, hearing problems, loss of vision</td>
<td>HSCT</td>
<td>250100#</td>
<td></td>
</tr>
<tr>
<td>Niemann–Pick A and B</td>
<td>Sphingomyelinase</td>
<td>CNS involvement, respiratory problems, gastrointestinal problems, hepatosplenomegaly</td>
<td>ERT⁎, SRT⁎</td>
<td>257200#</td>
<td></td>
</tr>
<tr>
<td>Gaucher</td>
<td>Glucocerebrosidase</td>
<td>CNS involvement, skeletal involvement, hepatosplenomegaly, hematologic manifestations</td>
<td>ERT⁎, SRT⁎ (II), HSCT⁎ (II)</td>
<td>230800#</td>
<td></td>
</tr>
<tr>
<td>Fabry</td>
<td>α-Galactosidase A</td>
<td>Renal and cardiac disease, cerebrovascular manifestations, pain/paresthesia, angiokeratoma, hypohidrosis</td>
<td>ERT, CT (II), CT⁎ ERT (II)</td>
<td>301500#</td>
<td></td>
</tr>
<tr>
<td>Krabbe, globoid cell leukodystrophy</td>
<td>Galactocerebrosidase</td>
<td>CNS and PNS involvement, hearing loss, gastrointestinal problems, loss of vision</td>
<td>HSCT</td>
<td>245200#</td>
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<tr>
<td>Mucolipidoses</td>
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<tr>
<td>Mucolipidosis</td>
<td>Lysosomal-enzyme N-acetylglucosaminyl-1-phosphotransferase</td>
<td>CNS involvement, dysostosis multiplex, cardiac disease, respiratory and ENT problems, hepatosplenomegaly, corneal clouding, hernias, macroglossia, gingival hyperplasia</td>
<td>HSCT** (II)</td>
<td>252500#, 252600#</td>
<td></td>
</tr>
<tr>
<td>Lipidoses</td>
<td>Niemann–Pick C</td>
<td>CNS involvement, liver failure, hearing loss, gastrointestinal problems, hepatosplenomegaly</td>
<td>SRT</td>
<td>HSCT (II/III)</td>
<td>257220#, 607625#</td>
</tr>
</tbody>
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<th>OMIM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wolman</td>
<td>Acid lipase</td>
<td>Gastrointestinal problems, hepatosplenomegaly, hematologic manifestations, adrenal disease</td>
<td>HSCT (II/III), ERT (II/III)</td>
<td>Cysteamine (II)</td>
<td>278000#</td>
</tr>
<tr>
<td>NCL 1</td>
<td>Palmitoyl-protein thioesterase-1</td>
<td>CNS involvement, loss of vision</td>
<td>Cysteamine (II)</td>
<td>256730#</td>
<td></td>
</tr>
<tr>
<td>NCL 2</td>
<td>Tripeptidyl peptidase-1</td>
<td>CNS involvement, loss of vision</td>
<td>GT (I/II)</td>
<td>204500#</td>
<td></td>
</tr>
<tr>
<td>Lysosomal transport defects</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cystinosis</td>
<td>Cystinosin</td>
<td>CNS involvement, myopathy, hepatosplenomegaly, renal disease, endocrine involvement, short stature, corneal clouding, loss of vision</td>
<td>Cysteamine</td>
<td>2,19800#, 2,19900#, 2,19750#</td>
<td></td>
</tr>
<tr>
<td>Glycogen storage disorder type II</td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pompe disease</td>
<td>α-Glucosidase</td>
<td>Myopathy, cardiac disease, respiratory problems, hearing loss, hepatomegaly, macroglossia</td>
<td>ERT</td>
<td>GT (I/II), CT (II), CT** ERT (I)</td>
<td></td>
</tr>
</tbody>
</table>

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led to the idea that lysosomal storage could be corrected by administering the missing enzyme. At that time, heterologous (endocytosis) was already known to deliver exogenous materials to the lysosomes [22].

ERT was first attempted in infants with Pompe disease through the intramuscular and intravenous administration of α-glucosidase from Aspergillus niger and human placenta, but had little effect [23]. However, the clearance of glycogen from the liver might now be seen as a first hint that the strategy could work [24]. All subsequent attempts at ERT in a broad variety of LSDs over the following decades had limited effect. In retrospect, this lack of effect was due to low doses, short treatment duration, sub-optimal enzyme preparations and the lack of knowledge about receptor-mediated endocytosis [25].

The existence of carbohydrate-specific receptors at the cell surface was first described by Ashwell and Morell [26], who discovered the role of the asialoglycoprotein receptor in hepatocytes, which bind glycoproteins with exposed galactose residues. Within a few years, endocytosis and lysosomal delivery had been shown to occur, according to cell type, through various receptors. Many cell types turned out to expose M6PRs on their surface, allowing the endocytosis of mannose 6-phosphorylated lysosomal enzymes [27,28]. Dendritic cells, liver Kupffer cells, and macrophages were found to expose mannose receptors, binding mannose-residue-rich sugar chains [29]. However, it was not until 1978 that the development of receptor-mediated ERT was actively pursued [30].

The first successful receptor-mediated ERT was achieved in Gaucher disease. In this case, human placental glucocerebrosidase was used as an enzyme source after partial deglycosylation with neuraminidase, galactosidase and hexosaminidase to expose mannose residues, to enable uptake by the mannose receptor of macrophages [31]. This successful attempt led to the registration of alglucerase in 1991, and revived belief in ERT as a potential therapy for LSDs. Its broad application came after the introduction of recombinant DNA technology, which allowed the large-scale production of lysosomal enzymes.

To date, ERT has been registered for Gaucher disease (imiglucerase [32], velaglucerase alfa [33] and taliglucerase alfa [34]), Fabry disease (agalsidase alfa [35] and agalsidase beta [36]), Pompe disease (agalactosidase alfa [37-39]) and MPS types I (laronidase [40]), II (idursulfase [41]) and VI (galsulfase [42]). ERTs for MPS IVA (recombinant human galactosamine-6-sulfate nafataze NCT01415427), α-mannosidosis (recombinant human α-mannosidase, NCT01285700), metachromatic leukodystrophy (MLD) (recombinant human arylsulfatase A, NCT00681811), Wolman disease (recombinant human lysosomal acid lipase, NCT01473875), and a second-generation ERT for Pompe disease (GILT-tagged recombinant human acid α-glucosidase, NCT01230801) are in different phases of clinical evaluation [43]. For LSDs other than Gaucher disease, the M6PR was chosen as the best target for efficient enzyme delivery.

### 2.1.2 Disease-related challenges

Although pivotal trials and clinical follow-up studies have demonstrated the beneficial effects of ERT, the overall outcome varies from disease to disease (Table 2). The effect of ERT depends first of all on the cell type primarily affected and on the accessibility of that cell type to ERT. Figure 2A shows that Kupffer cells, endothelial cells and hepatocytes in the liver have free access to the administered enzyme circulating in the blood. The accessibility of macrophages in the liver (Kupffer cells) and spleen contributes much to the success of ERT in Gaucher disease [44], and the accessibility of the Kupffer cells and hepatocytes to the effect on liver in MPSs. Another example is Fabry disease, where the location of the podocytes outside the glomerular filtration barrier prevents their effective treatment, whereas the endothelial cells lining the glomerular capillaries are treated effectively (Figure 2B). The blood–brain barrier (BBB) is a problem in its own right, as it prevents the access of the large therapeutic enzymes to the brain, which is clinically involved in many LSDs.

The effect of ERT is also determined by the molecular composition, the architecture and the natural turnover of the target tissue. For instance, through the continuous generation of macrophages in the bone marrow (BM) through differentiation of hematopoietic stem cells, the Gaucher cells are gradually replaced by enzyme-corrected macrophages. The bone problems in Gaucher disease are secondary to the storage of glucosylceramide in the Gaucher cells. By the time the Gaucher cells start to disappear, the bone infarctions appear to subside, but normalization of the bone structure – including bone density and Erlenmeyer flask shape – is delayed, most likely due to the slow turnover of bone.

The skeletal abnormalities in several of the MPSs (such as dysostosis multiplex) are also very difficult to correct, as they arise in part from abnormal cartilage anlage during fetal development and early post-natal life, and from storage of glycosaminoglycans (GAGs) in osteoclasts and osteoblasts [45]. The correction of GAG storage in the chondrocytes of the avascular articular cartilage and in the growth plate is very challenging, as the therapeutic enzyme will only diffuse through the matrix very slowly. Similarly, the cardiac valves are only slightly accessible to ERT. While microvasculature can be present in the heart valves, the myofibroblasts composing the valves are supplied with oxygen mainly by diffusion from the valve surface, and are therefore not easy targets for the relatively large therapeutic enzymes [46].

Animal studies have shown that some of the problems associated with cell and tissue accessibility may be overcome by increasing the dose of ERT [47,48]. In mice with Fabry disease, an effect on the heart was seen only with a dose of 10 mg/kg recombinant human α-galactosidase [47]. The therapeutic doses currently used in humans are 1 mg/kg for agalsidase alfa and 0.2 mg/kg for agalsidase beta. To optimize the therapeutic effect on cardiac and skeletal muscle, for instance, doses of up to 40 mg/kg of myozyme are now administered to patients with Pompe disease [37].
Table 2. Effects and challenges of approved ERTs in LSDs.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Enzyme replacement</th>
<th>Recommended dose</th>
<th>Main target organs</th>
<th>Effects observed in clinical trials</th>
<th>Main challenges §</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabry</td>
<td>Fabry Agalsidase alfa [35]</td>
<td>0.2 mg/kg/eow</td>
<td>Vascular endothelium, Kidney, Heart, PNS</td>
<td>Stabilizes renal function and reduces cardiomyopathy in mildly affected patients, reduces pain, improves quality of life</td>
<td>Renal and cardiac disease</td>
</tr>
<tr>
<td></td>
<td>Agalsidase beta [36]</td>
<td>1 mg/kg/eow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gaucher</td>
<td>Imiglucerase [32]*</td>
<td>1.5 mg/kg/eow</td>
<td>Liver, Spleen, Reticuloendothelial system, Bone, CNS</td>
<td>Improves blood counts, reduces hepatosplenomegaly, improves bone pain, some improvements in bone mineral density</td>
<td>CNS disease³ and skeletal pathology</td>
</tr>
<tr>
<td></td>
<td>Velaglucerase alfa [33]*</td>
<td>1.5 mg/kg/eow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Taliglucerase alfa [34]*</td>
<td>60 U/kg/eow</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MPS I</td>
<td>Laronidase [40]</td>
<td>0.58 mg/kg/week</td>
<td>Liver, Spleen, Connective tissue, Cartilage, Bone, CNS</td>
<td>Reduces hepatosplenomegaly, improves joint mobility, sleep apnea and pulmonary function, some improvements in walking distance in mildly affected patients</td>
<td>CNS disease, bone manifestations and corneal clouding</td>
</tr>
<tr>
<td>MPS II</td>
<td>Idursulfase [41]</td>
<td>0.5 mg/kg/week</td>
<td>Liver, Spleen, Connective tissue, Cartilage, Bone, CNS</td>
<td>Reduces hepatosplenomegaly, improves pulmonary function, walking distance and joint mobility</td>
<td>CNS disease and bone manifestations</td>
</tr>
<tr>
<td>MPS VI</td>
<td>Galsulfase [42]</td>
<td>1 mg/kg/week</td>
<td>Liver, Spleen, Connective tissue, Cartilage, Bone, CNS</td>
<td>Reduces hepatosplenomegaly, improves pulmonary function and walking distance</td>
<td>Bone manifestations and corneal clouding</td>
</tr>
<tr>
<td>Pompe</td>
<td>Alglucosidase alfa [37-39]</td>
<td>20 mg/kg/eow</td>
<td>Skeletal muscle, Smooth muscle, Heart</td>
<td>Prolongs survival, improves motor function and walking distance, stabilizes pulmonary function and reverses cardiac hypertrophy</td>
<td>Skeletal muscle weakness</td>
</tr>
</tbody>
</table>

*Indicated for patients with type I Gaucher disease.
³In Gaucher disease type II and III.
§ERT cannot pass the BBB.

BBB: Blood-brain barrier; CNS: Central nervous system; eow: Every other week; ERT: Enzyme-replacement therapy; LSD: Lysosomal storage disorder; PNS: Peripheral nervous system.
For personal use only.

Although, to a price of ERT is calculated per mg bodyweight, a higher dose more robust immunological response, though this has never dosing might prompt infusion-associated reactions or a that all ERTs are registered at a certain dose, and that higher shown that it is indisputably improved by higher doses. However, simply raising the dose is complicated by the fact that all ERTs are registered at a certain dose, and that higher dosing might prompt infusion-associated reactions or a more robust immunological response, though this has never been demonstrated.

A second consideration in this respect is that because the price of ERT is calculated per mg bodyweight, a higher dose automatically increases the cost of treatment. Although, to a certain extent, this can be remedied by reducing the cost of treatment through the development of less expensive production systems such as genetically modified yeast or carrots or transgenic animals (milk), production accounts for only part of the total cost, which also includes development, registration and marketing. For rare disorders, these costs are extremely high.

Recently, human recombinant glucocerebrosidase produced in genetically modified plant cells (taliglucerase alfa) has been approved for the treatment of Gaucher disease [34]. Although the very first initiatives toward producing recombinant human acid α-glucosidase in the milk of transgenic rabbits were stopped for strategic reasons, 6 of the 7 patients with Pompe disease who participated 12 years ago in a Phase II clinical trial involving recombinant human acid α-glucosidase from rabbit milk are still alive, and are currently treated with alglucosidase alfa.

Irrespective of production costs, another way of improving the effect of ERT beyond increasing the dose might be by ameliorating the uptake of ERT by the target cells. To this end, the enzyme can be conjugated with synthetic oligosaccharides bearing M6P. This is envisaged for Pompe disease (oxime-neo-recombinant human α-glucosidase). The artificial product was shown to clear the lysosomal glycogen storage in muscle of immunotolerized Pompe mice better than the unmodified enzyme [52].

The application of GILT technology (glycosylation-independent lysosomal targeting) may be another way of improving the effect of ERT in Pompe disease. GILT involves the replacement of the N-terminal propeptide of acid α-glucosidase by an N-terminal fragment of insulin-like growth factor 2 (IGF-2) that includes the signal peptide [53]. The IGF-2 propeptide binds with high affinity to the M6PR. The fusion protein composed of IGF-2 and recombinant human acid α-glucosidase is currently being tested in a Phase II clinical trial (NCT01230801) [43]. In Pompe mice, the glycogen clearance effect of the fusion protein was generally equivalent to a corresponding fivefold higher dose of alglucosidase alfa [53].

To improve uptake in the central nervous system (CNS), recombinant proteins are being coupled to a monoclonal antibody against an endogenous BBB receptor, such as the human insulin receptor or the transferrin receptor [54]. This has increased enzyme uptake in the brain of MPS II animal models [55].

The uptake of recombinant enzymes might also be improved through modulation of the M6PR expression on the target cell. Adjunctive treatment of Pompe mice with clenbuterol, a selective β2-agonist, enhanced the M6PR expression in skeletal tissue and increased efficacy of ERT as measured by motor function and glycogen clearance [56].

Enzyme uptake in specific organs may be improved by local enzyme injection. Injection of enzyme into the intracerebrospinal fluid in animal models has reduced neuropathology and improved neurological function [57].

### 2.1.3 How can the effect of ERT be improved?

Several strategies for enhancing the effect of ERT are now being explored. In vitro studies and animal studies have shown that it is indisputably improved by higher doses. However, simply raising the dose is complicated by the fact that all ERTs are registered at a certain dose, and that higher dosing might prompt infusion-associated reactions or a more robust immunological response, though this has never been demonstrated.

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Enzyme uptake in specific organs may be improved by local enzyme injection. Injection of enzyme into the intracerebrospinal fluid in animal models has reduced neuropathology and improved neurological function [57].

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**Figure 2. Accessibility of cell types to circulating recombinant enzymes.** A. The Kupffer cells (K), endothelial cells (E) and hepatocytes (H) in the liver have free access to the administered enzyme-replacement therapy. B. In the kidney, the endothelial cells (E) have direct access to the administered enzyme, whereas the location of the podocytes (P) outside the glomerular filtration barrier prevents their effective treatment.
In humans, such a strategy poses several ethical and practical questions. Intrathecal administration of laronidase in a patient with MPS I seemed safe and appeared to alleviate some signs and symptoms of spinal cord compression [58]. The safety and efficacy of intrathecal application of ERT is currently evaluated in open-label Phase I/II studies in MPS IIIA (recombinant human heparan N-sulfatase, NCT01299727), MLD (recombinant human arylsulfatase A, NCT01510028) and MPS I (laronidase, NCT00852358) [43]. Other clinical studies currently investigate the safety of combined intrathecal and intravenous idursulfase administration in MPS II (NCT01506141), and the administration of intrathecal idursulfase around the time of HSCT in MPS I (NCT00638547) [43].

All forms of ERT are complicated by their burden on patients, who need to receive it life-long, at least twice a month. And, as stated above, ERT is also expensive: its annual cost can amount to US$145,000 – 377,000 per patient [59].

2.2 Hematopoietic stem-cell transplantation

Although most LSDs do not result in hematopoietic defects, allogeneic hematopoietic stem-cell transplantation (HSCT) might offer an effective treatment. Through infiltration into various tissues, healthy donor cells can replace enzyme-deficient cells. In theory, they can serve as a permanent source of the missing enzyme, and correct neighboring cells through enzyme secretion and uptake by enzyme-deficient host cells. Various graft sources can be used, including BM, peripheral blood (PB) and cord blood (CB).

In 1980, the first allogeneic HSCT was performed in a patient with MPS I, who received BM from a human leukocyte antigen (HLA)-matched relative. He showed biochemical as well as clinical improvements for up to 13 months after transplantation; these included a reduction in hepatosplenomegaly and corneal clouding, and improvement in growth, development and cognition [60].

Although HSCT has since been attempted in many of the LSDs [61], its application is currently mainly limited to a subset of patients with MPS I (Hurler), Krabbe disease and metachromatic leukodystrophy (MLD) [62]. The clinical effects in MPS I (Hurler) seem the most rewarding.

Hematopoietic stem cells differentiate into white blood cell lineages and migrate to several organs in mice, such as BM, liver (Kupffer cells), spleen, lung (alveolar macrophages) and the CNS (microglia) [63]. However, they barely home in tissues such as skeletal muscle and cartilage, as shown in rats [64]. In patients with MPS I, HSCT is effective in reducing hepatosplenomegaly, improving airway obstruction and cardiac function, it can improve corneal clouding, hearing and psychomotor development, and improves survival. Although it can also improve joint mobility, it does not correct skeletal manifestations, and cardiac valvular deformities appear to resolve poorly [65]. Although one might have expected HSCT to have similar effects in other MPSs, a limited number of studies have shown less beneficial effects. This may be related to the fact that patients with other MPSs were usually transplanted at a later age, or to the use of other graft sources or conditioning regimens [62]. In MPSs with minimal neurological involvement, such as MPS VI, the possible benefits of HSCT have to be weighed against the risks.

Since it is biologically easier to prevent pathology than to correct it, the effect of HSCT is bound to depend on the disease status at the time of transplantation. Transplantation should therefore take place at a very young age, preferably before the clinical symptoms manifest themselves. This is particularly important for preventing irreversible damage in the CNS: as microglia turnover is very slow, engraftment takes a considerable time [63]. An example of this was reported in Krabbe disease: although CB transplantation improved neurological and developmental outcome in asymptomatic newborns, minimal neurological improvement took place in symptomatic infants [66]. In 2006, these findings and considerations underlay the start of a screening program for Krabbe disease in New York State [67]. However, the long-term effects of presymptomatic children transplanted for Krabbe disease are not optimal; progressive neurologic deterioration is still observed in many of the treated patients [68].

The serious complications of HSCT are graft failure and procedure-related risks of mortality and long-term morbidity. To limit these risks, careful selection of a HLA-matched donor graft source and conditioning regimen used to eliminate the patient’s own stem-cell population is required. In MPS I, the implementation of transplantation guidelines has significantly improved event-free survival (defined as alive and engrafted) [62]. Similarly, the availability of banked CB stem cells has reduced the time to transplantation. CB has several other advantages, including reduced graft-versus-host disease despite a higher level of HLA incompatibility, the likelihood of sustained engraftment in MPS I and the suggested capacity of multipotent stem cells in CB to differentiate into osteoblasts, chondroblasts and neurons [69], as a result of which CB might be considered as the preferred cell source in HSCT for LSDs [70].

As HSCT results in partial correction of the clinical manifestations, and as hematopoietic stem cells do not engraft well in all tissues, the use of alternative donor cell sources is being explored. For example, to reduce residual disease in patients with MPS I and MLD who previously underwent successful HSCT, mesenchymal stem cells, capable of differentiation into a large variety of tissues, were infused, but the clinical effect was limited [71]. The ability of human neural progenitor cells to participate in brain repair has recently been demonstrated in several preclinical studies [72]. One Phase I clinical trial has been completed using human CNS stem cells directly implanted into the brains of children with infantile or late infantile neuronal ceroid lipofuscinosis (NCL); results are being awaited (NCT00337636) [43].

In practice, ERT is often used to optimize the patient’s condition in anticipation of HSCT engraftment [73]. Stem-cell therapy can also be combined with gene therapy (see Section 2.3).
2.3 Gene therapy

Gene therapy is based on the idea of transferring DNA that encodes a functional enzyme into the patient’s enzyme-deficient cells so as to correct the enzyme deficiency and provide a potentially permanent source of therapeutic enzyme. The level of expression can be manipulated by using strong promoters. Since LSDs are monogenic disorders, and low enzyme activities are often sufficient to bring clinical improvement, LSDs are good candidates for gene therapy.

Gene transfer can be applied in vivo and ex vivo. For in vivo gene therapy, a vector carrying the transgene is injected either into the circulation or directly into a target tissue (e.g., the brain). ex vivo gene therapy implies correction of the patient’s cells by genetic modification in vitro, followed by re-implantation. The use of genetically modified hematopoietic stem cells makes it possible to achieve migration of corrected cells into organs such as the brain, which are otherwise hard to access [74]. Since ex vivo gene therapy entails autologous cells, transplant-related mortality and morbidity is lower than in allogeneic HSCT. ex vivo gene therapy can also involve the use of organoids (artificial organs) [75].

The feasibility of gene therapy has been demonstrated in various studies performed in a range of animal models. Both in vivo and ex vivo gene therapy can lead to biochemical and clinical improvements [76,77]. As for ERT, these studies have shown that transduction in certain tissues (such as brain) is more difficult than in others (such as liver). In addition, the effect of gene therapy depends on the level of transgene expression, which in turn determines how much therapeutic enzyme is secreted by the genetically corrected cells and potentially available for correction of more distant tissues and organs [77]. Although the outcome of gene-therapy studies in animal models has been fairly promising, many safety and efficacy issues need to be addressed for application in humans.

One major obstacle to the clinical application of gene therapy is the potential risk of oncogenesis due to undesirable integration of the transgene construct into the genome. Attempts at gene therapy for patients with X-linked SCID using retroviral vectors led to successful treatment of 9 of 10 patients, but also led to lymphoproliferative disorders in four of these patients [78,79]. Viral vectors also risk eliciting inflammatory and immunological responses; the latter may increase with readministration [80]. The transgene product may also elicit a humoral immune response, which can reduce the effect of gene therapy. Restriction of enzyme expression to the liver can reduce this immune response, presumably by reducing expression of the transgene in antigen-presenting cells [80].

The key issue in somatic gene therapy is finding the right vector for the delivery and long-term expression of the transgene in the target tissues. For gene transfer in LSDs, mainly viral vectors are used, particularly adeno-associated viruses (AAV) and lentiviruses [81]. These vectors can infect a wide range of tissues, infect dividing and non-dividing cells and induce long-term transgene expression. Whereas lentiviruses insert into the genome, and can thus induce insertional mutagenesis and oncogenesis, present-day AAV vectors are essentially episomal and are non-pathogenic.

Thus far, about 20 clinical trials have been performed with gene therapy in LSDs, but as yet none has reached Phase III [81]. Recently, it was suggested that administration of an AAV serotype 2 vector expressing the human CLN2 cDNA into the brain of children with late infantile NCL (Batten disease) slowed neurological progression in the treated children compared with untreated controls [82]. A follow-up study is currently ongoing (NCT01414985) [43]. Phase I/II clinical trials are currently being performed for Pompe disease (NCT00976352) to evaluate the delivery of a recombinant AAV acid α-glucosidase gene vector to the diaphragm, and for MLD (NCT01560182) using gene-modified HSCT [43]. Direct intracranial injection of viral gene vectors has resulted in reduced lysosomal storage and functional improvement in some large animal models of LSDs [76]. A Phase I/II trial evaluating the tolerance and safety of intracerebral administration of an AAV vector carrying the N-sulfoglucoamin sulfohydrolase and sulfatase-modifying factor I cDNAs for the treatment of MPS IIIA is currently ongoing (NCT01474343) [43].

As well as viral vectors, non-viral vectors are being developed as an alternative method of gene delivery and targeting. Methods of delivery include the encapsulation of DNA in polymers, liposomes or nanoparticles [81], which may provide vehicles to cross the BBB [83].

If the vectors and procedures become safer, more specific and more effective, gene therapy is likely to become applicable in clinical practice.

2.4 Chaperone therapy

During co-translational import into the ER, missense mutations in genes coding for lysosomal proteins often lead to improperly folded or unstable proteins. These proteins are either recognized by the ER quality-control system (ERAD), and then exported and degraded by proteasomes; or they continue their journey to the lysosomes, but fail to reach their final destination. The aim of chaperone therapy is to reduce the elimination of these proteins, which can be catalytically active. Chaperones are small molecules that selectively bind and stabilize target proteins, thereby mimicking normal folding, improving intracellular trafficking and increasing lysosomal enzyme activity. While chaperone therapy is expected to restore small conformational changes, it is not expected to restore the effect of mutations that cause major structural changes (e.g., null-mutations or frameshift mutations). The chaperones currently used or developed for the treatment of LSDs are reversible competitive inhibitors of their target enzyme.

Many in vitro studies have shown that a growing number of chaperones can restore enzyme transport and maturation, and increase residual enzyme activity in cells of patients with chaperone-sensitive mutations [84].
The first clinical trial of chaperone therapy for Fabry disease using galactose claimed remarkably good results in a patient with the cardiac variant of Fabry disease [85]. Galactose was administered intravenously every other day, whereas the current chaperones have the advantage of oral administration.

Several Phase I/II clinical trials have been conducted for Fabry (migalastat hydrochloride) and Gaucher disease (isofagomine), and GM2 gangliosidosis (pyrimethamine). Some patients were reported to respond positively to these chaperones, by increased enzyme activity levels in leukocytes [86,87]. Communications from the manufacturer reported that the renal function of responding Fabry patients remained stable during 2 – 3 years of treatment [87]. In Gaucher disease, clinically meaningful improvements in key measures of disease were reported in only 1 of the 18 patients after 6 months of treatment [87]. In GM2 gangliosidosis, clinical effects were difficult to assess, mainly due to short treatment duration of up to 10 months and inter-individual variability [86]. Treatment was generally well tolerated in Fabry and Gaucher disease [87]. In GM2 gangliosidosis, significant side effects were experienced by most patients at or above 75 mg pyrimethamine per day [88]. A Phase II clinical trial in Pompe disease with 1-deoxynojirimycin hydrochloride was suspended due to serious and probably treatment-related adverse events (NCT00688597) [43,89].

At present, a randomized, placebo-controlled Phase III clinical trial with chaperone therapy is ongoing for Fabry disease (migalastat hydrochloride, NCT00925301), and an uncontrolled open-label, Phase II extension study is ongoing for Gaucher disease (isofagomine, NCT00813865) [43].

Whereas the first chaperones were identified by rational drug design, recently, high-throughput screening of libraries with chemicals and registered drugs has been successfully utilized to identify novel chaperones. This strategy led to the identification of the drug ambroxol as a potential chaperone for Gaucher disease [90]. Ambroxol was demonstrated to increase the activity of glucocerebrosidase in cultured fibroblasts from patients and in wild-type mice [90,91]. An open-label Phase I/II clinical trial has been announced to assess its safety and proof-of-concept (NCT00433147) [43].

The continued interest in chaperone therapy is explained largely by their potential to target many organs and to cross the BBB. This has been illustrated in mouse models for Fabry [92], Gaucher [91,93], GM1 gangliosidosis [94] and Pompe disease [84]. Administration of N-octyl 4-epi- β-valienamine in GM1 gangliosidosis mice starting at the early stage of disease resulted in remarkable arrest of neurological progression within a few months, and prolonged survival [94]. Other advantages of chaperones are that they can be administrated orally, making therapy minimally invasive, and that these small molecules are relatively easy to produce, which reduces their cost. Neither is antibody formation likely to occur.

A real disadvantage of chaperone therapy is that their use is restricted to a small subset of mutations, as shown in Fabry [95], Pompe [96] and Gaucher disease [97], and in GM1 gangliosidosis [98]. Second, as the current generation of chaperones competes with the endogenous substrates for binding to the active site of the lysosomal enzymes, dosing is very critical. The chaperone must correct protein folding as much as possible and inhibit the catalytic function as little as possible. To optimize the effect, dose optimization studies have been performed in animal models. For example, in Fabry mice that express a mutant form of human α-galactosidase A (R301Q) on a knockout background, intermittent administration of chaperone therapy (4 days on/3 days off or every other day) resulted in greater substrate reduction than daily administration [92]. Another approach to circumvent the competition between chaperones and endogenous substrates is to search for chaperones that bind to alternative sites. For this purpose, novel high-throughput screening methodologies have been used to identify non-inhibitory chaperones for Gaucher disease [99] and to search for chaperones that act as enzyme activators in Pompe disease [100].

It is being explored whether chaperones can have a synergistic effect when combined with ERT. Compared with ERT alone, co-administration of chaperones with ERT has been shown to enhance substrate clearance and increase cellular enzyme activity in Gaucher [101], Fabry [102] and Pompe [103] disease in vitro, and in Fabry [104] and Pompe mice [105]. Drug–drug interaction studies of chaperone therapy and ERT in patients with Fabry (NCT01196871) and Pompe disease (NCT01380743) are currently ongoing, and the results of a study in patients with Gaucher are awaited (NCT00433147) [43].

### 2.5 Substrate-reduction therapy

The objective of SRT is to minimize lysosomal storage by inhibiting the synthesis of the substrate and thereby improving the balance between lysosomal input and degradation. In this way, the low level of residual enzyme activity, usually found in patients with slowly progressive phenotypes, may become sufficient.

As glucosyltransferase is a key enzyme in the synthesis of glycosphingolipids, it was proposed that inhibition of this enzyme could reduce the production of glycosphingolipids [105]. The first iminosugar used for this purpose was N-butyldeoxynojirimycin (miglustat), which reversibly inhibits ceramide-specific glucosyltransferase and shows structural similarities with active site-directed inhibitors such as chaperones.

The administration of N-butyldeoxynojirimycin to adults with Gaucher disease type I reduced hepatosplenomegaly and improved blood counts and disease biomarkers [106]. In 2003, these results led to the award of marketing approval for mild to moderately affected patients with Gaucher disease type I for whom ERT is not a therapeutic option, for example, because of persistent side effects to ERT. Longer follow-up studies have shown significant improvements in all major efficacy end points, and have demonstrated effectiveness over time [107]. Although N-butyldeoxynojirimycin has been
shown to cross the BBB in several mouse models, it showed no improvement in neurological symptoms over a 2-year period in patients with Gaucher disease type III [108]. In a sibling study in two adults with saposin C deficiency, N-butyldeoxynojirimycin had no significant effect after 2 years of treatment [109].

In 2009, N-butyldeoxynojirimycin was also approved for the treatment of Niemann–Pick disease type C (NPC), as it had been shown to stabilize or improve saccadic eye movements, a neurological symptom of NPC in a randomized controlled study [110]. The mechanistic action of N-butyldeoxynojirimycin in NPC, in which glycolipids and cholesterol accumulate, is not fully clear. It might act through a direct effect on lipid trafficking or indirectly by modulating intracellular calcium homeostasis or reduction of oxidative damage [111].

N-Butyldeoxynojirimycin causes side effects consisting of gastrointestinal problems such as flatulence, bowel cramps and diarrhea, which is probably caused by inhibition of the intestinal carbohydrate-digesting enzyme sucrase-isomaltase [112]. Another inhibitor acting on glucosyltransferase is eliglustat. Although the biochemical and clinical results obtained with eliglustat were similar to those obtained with N-butyldeoxynojirimycin [113], its higher specificity for glucosyltransferase and the absence of inhibition of intestinal glucosidases, meant that the treatment-related gastrointestinal side effects were minimal [114]. Eliglustat is currently being evaluated in a Phase III randomized, double-blind, placebo-controlled trial in patients with Gaucher disease type I (NCT00891202) [43].

Genistein is a potential SRT in the MPSs. Genistein is a soybean isoflavone that has non-specific glycosaminoglycan-reducing ability. In MPS II [115] and MPS IIIB mice [116], it has been shown to reduce GAG accumulation in non-CNS tissue. In an MPS IIIB mouse model, it had various effects on neuropathology; most notably, it reduced heparan sulfate storage in the brain, improved synaptic function and corrected behavior abnormalities [117].

Although the first clinical trial using genistein in patients with MPS IIIA and B for 12 months showed statistically significant improvements in urinary GAG concentration, hair morphology and cognitive function [118], a recent placebo-controlled cross-over study showed that genistein had little effect in MPS III patients [119]. Total GAGs in urine decreased significantly, but there were no measurable effects on overall behavior or hair morphology. Furthermore, neither parents nor caregivers were able to distinguish the periods in which the patients received either treatment or placebo.

In summary, SRT seems a promising approach for treating some of the LSDs. Advantages include the potential for crossing the BBB, lower production costs than ERT and oral administration. Conceivably, improvements will be achieved by searching for novel compounds with higher specificity and improved range of action.

2.6 Other pharmacotherapeutic options

2.6.1 Cysteamine bitartrate
In 1994, cysteamine bitartrate was approved by the US Food and Drug Administration as a therapy for cystinosis, an LSD in which cystine accumulates due to deficiency of the cystine transporter. Cysteamine bitartrate enters the lysosome and reacts with cysteine to form cysteine and cysteine–cysteamine complexes, which can leave the lysosome [120].

In 2006, Lu and Hofmann suggested that cysteamine might be useful for the treatment of infantile NCL 1, since cysteamine acts on thioether linkages and seems to cleave palmitoyl-CoA (coenzyme A), which is the accumulating substrate in NCL 1. Cysteamine bitartrate resulted in a modest reduction of palmitoyl-CoA accumulation in isolated lymphoblasts of NCL 1 patients [121]. Cysteamine bitartrate, alone and in combination with N-acetylcysteine, is currently being tested for the treatment of NCL 1 (NCT00028262) [43].

2.6.2 Nonsense mutation suppression
Some patients with LSDs have nonsense mutations that prematurely abort translation and give rise to a truncated protein, usually a non-functional one. In such cases, therapy can be targeted on suppressing the nonsense mutation with drugs [122] such as ataluren, which has been shown to increase enzyme activity in cultured fibroblasts of patients with infantile NCL [123]. Ataluren is a non-toxic compound that promotes ribosomal read-through of premature stop codons caused by nonsense mutations. The compound is supposed to prevent translation termination and to stabilize nonsense-containing transcripts, thus enabling quasi-normal enzyme formation. Ataluren has had positive effects in patients with cystic fibrosis caused by nonsense mutations [124]. Though its clinical effects to date have been limited, this therapeutic approach is currently being evaluated in an uncontrolled open-label Phase III clinical trial for the treatment of cystic fibrosis (NCT01140451) [43].

2.6.3 Targeted gene repair
Targeted gene repair can be seen as the ultimate art of gene therapy. It is intended to correct specific point mutations through the use of site-specific oligonucleotides [125]. Whatever its promise, it has not yet shown its potential value in clinical practice: targeted correction of a single-base change using chimeric RNA/DNA oligonucleotides in a model system employing Gaucher fibroblasts produced no genomic correction [126].

3. Expert opinion

Much progress has been made in the development of therapeutic strategies for LSDs. In the past decades, several therapies have been brought to market and have significantly improved the outcome of patients with LSDs. Unfortunately, none of the current therapies are fully curative yet. Interference with the pathologic process either has to start with cellular...
repair through any therapeutic means, or by replacing affected cells through stem-cell therapy. In all instances, the timing of the start of treatment is crucial, as tissue and organ damage become irreversible on disease progression.

What prevents the current therapies from being fully curative is the multi-organ involvement in LSDs caused by the ubiquity of lysosomes in all cell types other than red blood cells; this requires the correction of many specialized cell and tissue types, which is difficult for ERT and gene therapy to target. While some tissue types seem to require a high dose of circulating enzyme, this automatically results in higher costs. A potentially useful feature of gene therapy is that it can be directed to a distant organ, for instance, the liver to make it serve as permanent enzyme reservoir. The tightness of the BBB impedes correction of CNS pathology by ERT.

The BBB problem is partially circumvented by chaperone therapy and SRT. Their use is of limited applicability, as their effect depends on the patient’s genetic mutation. These therapies are further complicated by their narrow therapeutically effective range: while too high a dose of chaperone therapy may suppress the enzyme’s activity, as the chaperones currently available settle in the active site pocket, too low a dose is ineffective. Too high a dose of SRT risks harmfully inhibiting essential biosynthetic pathways.

New avenues to therapeutic intervention might be opened by greater insight into the pathophysiological processes leading to the complex phenotypes of LSDs. Currently, the knowledge is still scarce on how the lysosomal protein dysfunction caused by lysosomal gene defects eventually results in the clinical phenotype, and on the extent to which genetic, cell-type-specific and environmental factors play a role in this process. Knowledge of these processes might prompt the development of novel therapeutic targets.

With regard to the near future, it is believed that the therapeutic effectiveness of ERT can be improved and costs be reduced by intelligent design of the therapeutic enzymes, for example, by maximizing their M6P content or make them bind with higher affinity to the bifunctional M6P/IGF-2 receptor (GILT technology) [53]. Costs might be reduced through alternative production methods such as the use of plant cells to produce recombinant human glucocerebrosidase for the treatment of Gaucher disease [34], or yeast-cell systems to produce therapeutic enzymes for GM2 gangliosidosis [127]. In the past, production of acid α-glucosidase in rabbit milk and larger transgenic animals proved very efficient and therapeutically effective; its cost is potentially low [128]. In cases in which antibody formation clearly counters the effect of treatment, improvement can be achieved by eliminating the immune response [50].

In the near future, once safe vectors with proper specificities have been developed, enzyme therapy will probably be replaced by the in vivo or ex vivo use of gene therapy. In the meantime, the search for modifying factors may lead to the identification of new therapeutic targets that can help to improve the response to existing methods. Modifying factors might then support the selection of the best therapy for individual patients.

In the more distant future, stem-cell therapy may provide a tool for local tissue replacement. Natural stem-cell sources and induced pluripotent stem cells (iPS cells) are currently being considered for this purpose [129]. In theory, iPS cells have great potential, since they can be stimulated to differentiate into various lineages including neuronal stem cells [130,131]. The cells can be derived from the patient and then genetically corrected before re-transplantation. This technology avoids the ethical dilemmas inherent to obtaining stem cells, and also circumvents robust immunological responses.

It is crucial to the success of any type of therapy – existing or potential – that therapy starts before the disease process has caused irreversible tissue damage. Although precious time in this respect can be gained by neonatal screening programs [132], they raise ethical questions [133]. Most LSDs are characterized by a very broad clinical spectrum, and many patients will be diagnosed long before their first disease symptoms manifest. Patients are then condemned to live with the burden of a disease that may not develop until late adulthood.

Clinical trials in LSDs are complicated by the rarity of the diseases, and the broad spectrum of phenotypes within each disease [8]. This demands that knowledge is preferably gathered and shared by Centers of Expertise. To investigate the effect of therapeutic interventions and to choose the best clinical endpoints for future trials, this process should start by delineating the natural course of the disease.

In conclusion, considerable progress has been made in establishing different therapies for patients with LSDs. As the number of studies is continuously expanding, the first fully curative treatment for LSDs is likely to become a reality within the next few decades.

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Declaration of interest

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the Netherlands. This agreement also caters to financial support for Erasmus MC for research in Pompe disease. Erasmus MC and inventors for the method of treatment of Pompe disease by enzyme-replacement therapy receive royalty payments pursuant to Erasmus MC policy on inventions, patents and technology transfer.

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