Emerging therapies and therapeutic concepts for lysosomal storage diseases

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Introduction: The success of the first enzyme replacement therapy (ERT) for a lysosomal storage disease (LSD) and the regulatory and commercial incentives provided by authorities for orphan and rare diseases has spawned a massive interest for developing drugs for these intriguing but devastating genetic disorders. The potential for new drugs in this arena is vast, as not only a high number of LSDs have no available therapy, but also alternative therapeutic approaches for diseases with existing treatment are much needed as a number of challenges facing the existing therapies have become very obvious. A significant unmet medical need is therefore apparent for most, if not all of the LSDs and the development of new therapies based on the increasing knowledge of the pathophysiological mechanisms involved in these devastating diseases is therefore anticipated with great interest from all stakeholders.

Areas covered: The reader will be introduced to the intricate biological processes involved in lysosomal regulation and how these are exploited for current and emerging therapies. Therapies utilizing these processes will be thoroughly reviewed with regard to their mechanism of action, their clinical status and the challenges they are faced with and/or are aiming to address. For this review, a literature research has been undertaken that covers the years 1955 – 2012.

Expert opinion: The interest in lysosomal biology and disease has surged over the past decade not only in the halls of science but also of pharmaceutical companies. As the complexity of the LSDs increasingly become revealed, so do novel therapeutic targets continuously nurturing the development of new candidate drugs for these devastating diseases. Among this multitude of approaches, the ERTs still account for the vast majority of approved therapies but a number of exciting alternative approaches are emerging targeting various components of the pathophysiological cascade. This evolution of the field is much needed as the presently available treatments are unable to address all clinical aspects of these multifaceted diseases. Future therapy will most likely consist of combinations of these established and emerging approaches as well as other yet to be discovered concepts as the complexity of the diseases demands a certain degree of humbleness to the expectations for a cure based on a single therapy.

Keywords: chaperone, enzyme replacement, Fabry disease, Gaucher disease, glycosphingolipids, lysosomal storage disease, lysosomes, substrate optimization, substrate reduction, therapy

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1. Background

Since the discovery and initial characterization of lysosomes by Francois Appelmans, Robert Wattiaux and Christian de Duve in 1955 [1-3], the interest for this still enigmatic organelle and its close relatives has seen a resurgence in the past
two decades spurred by the rediscovered and increasing realization of their crucial roles in physiological homeostasis. The interest in lysosomes from not only academia but also biotech and pharma has been further kindled by a number of clinical, regulatory and commercially transforming events that have provided a new paradigm for developing therapies for not only lysosome-related diseases but also other rare and orphan diseases.

With the introduction of the Orphan Drugs Act in the USA in 1983, which was followed by similar legislation in Singapore (1991), Australia (1993), Japan (1997) and the EU (1999), a regulatory framework has been laid down for guiding the development of drugs for rare diseases with highly unmet need. However, the commercial potential of developing drugs for lysosomal storage diseases (LSDs) was not realized until the development of Cerezyme® (recombinant glucosylceramidase (glucocerebrosidase) for the treatment of type I Gaucher disease) by the Boston-based biotech company Genzyme.

The scientific rationale for the development of lysosome-targeted therapies however predates the advent of Cerezyme by several decades and driven by the re-kindled interest in lysosomal diseases several novel therapeutic concepts and therapies are now emerging for these devastating disorders.

1.1 Lysosomes

As the main compartment for intracellular degradation and subsequent recycling of cellular constituents, the lysosomes receive both hetero- and autophagic cargo, which in the lumen of this multifaceted organelle find their final destination. The degradation is carried out by a number of acid hydrolases (glycosidases, proteases, sulfatases, lipases, etc.) capable of digesting all major cellular macromolecules [4]. These acid hydrolases function optimally at the acidic pH of the lysosomes (pH 4 – 5) although several can still function and have distinct roles at the neutral pH outside the lysosomes, albeit having decreased stability and/or altered specificity [5].

Until recently, the function of many of these enzymes was thought to be limited to intralysosomal macromolecule turnover. However, from the complex and diverse clinical presentation of the diseases originating from lysosomal malfunction and involvement in neoplastic events it is clear that the lysosomes have an absolutely critical role in physiological homeostasis [6,7]. As such, the potential impact of therapeutically addressing the lysosomes and their constituents should not be underestimated.

Interestingly, recent data suggest that the biogenesis and functioning of endosomal and autophagosomal pathways is partially controlled by the transcription factor EB (TFEB), which regulates a coordinated lysosomal expression and regulation (CLEAR) gene network [8], a finding which argues for an evolutionary need to intimately control and efficiently adapt the lysosomal system to rapid changes in the cells metabolic state.

1.2 Dynamics of the lysosomal system

The intracellular trafficking of vesicles involved in, or related to, the lysosomal system, serve an essential role in the mammalian cell through its delivery of membrane components, various solute molecules and receptor-associated ligands to a range of intra- and extracellular compartments. The main pathways involved in this system are depicted in Figure 1, along with highlights of the various facets of these pathways being targeted for therapeutic intervention in the LSDs (Figure 1).

As a testament to the importance of this system and its constituents, defects in any part of it leads to a number of severe diseases or syndromes. Be it defects in lysosomal exocytosis (e.g., Chediak–Higashi syndrome), reduced lysosomal catabolic efficacy (e.g., Niemann–Pick disease types A and B and Gaucher disease), lysosomal transport machinery defects (e.g., Griscella syndrome and Charcot–Marie–Tooth disease), lysosomal metabolite efflux impairment (e.g., Niemann–Pick type C and cystinosis/Fanconi syndrome) or dysfunction of lysosomal integral membrane proteins (e.g., Danon disease), the disease most often affect multiple organs and tissues, involves the central nervous system (CNS) and is often fatal at a young age in its aggressive forms [7,9-13].

In order to understand the complexity of the LSDs and why defects in this refined machinery can lead to such detrimental clinical manifestations as well as grant the reader an initial overview of the possibilities that might exist for therapeutic intervention, a brief description of the inter-relations in the lysosomal system is provided below.

1.2.1 Endocytic route to lysosomes

The best understood endocytic pathway, which is also extensively exploited in enzyme replacement therapies (ERTs) for the LSDs, is the receptor-mediated endocytosis of molecules via the formation of clathrin-coated pits [14]. In the conventional receptor-mediated endocytic pathway, receptors such as the transferrin receptor, the low-density lipoprotein receptor and the mannose 6-phosphate receptor (M6PR) concentrate into clathrin-coated pits on the surface of the plasma membrane and form early endosomes [15,16]. Although the majority of lysosomal enzymes are targeted to the lysosomes from the trans-Golgi network (TGN) through mannose-6-phosphate (M6P)-mediated binding to M6PRs in the medial Golgi and then released once the Golgi-derived transport vesicles fuse with late endosomes (detailed in Section 1.2.2), some amounts of lysosomal enzymes are destined for secretion and subsequent uptake from the extracellular space via plasma membrane receptors that are either the same or similar to those involved in intracellular sorting and the biosynthetic route to the lysosomes [15]. This concept developed from a number of metabolic complementation studies in cells from patients with different LSDs and is the fundamental principle for enzyme therapies of LSDs [17].

As the endosome mature, its luminal pH steadily drops, mainly through the action of the vacuolar-type proton
ATPase (V-ATPase) [18], facilitating the dissociation of receptor and ligand while shifts in membrane lipid and protein composition also occur as the vesicles mature to form late endosomes and subsequently lysosomes. The late endosomes and lysosomes differ from endosomes primarily in their degree of acidification, higher buoyant density, higher abundance of integral lysosome-associated membrane proteins (LAMPs) and enrichment of acidic hydrolases [19].

1.2.2 Biosynthetic route to lysosomes

Apart from endocytosis, late endosomes and lysosomes also receive cargo via the M6PR pathway from the TGN (the biosynthetic route). The cation-dependent M6PR and the cation-independent (CI) M6PR/insulin-like growth factor-II (IGF-II) receptor share the task of delivery of newly synthesized acid hydrolases from the TGN to the lysosomes. The recognition of acid hydrolases by M6PRs requires the addition of carbohydrates in the endoplasmic reticulum (ER) and the subsequent modification and phosphorylation of the carbohydrate residues to M6P moieties in the cis-Golgi [15,20]. The M6PR-bound hydrolases are first delivered to endosomes, where they dissociate from the receptors due to the drop in the lumenal pH, hereby allowing the receptors to recycle back to the TGN.

1.2.3 Autophagic routes to lysosomes

Autophagy is the third relatively well-characterized route by which macromolecules reach the lysosome. Autophagy is an

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**Figure 1. Lysosomal pathways.** The figure provides a general overview of the dynamics of the endolysosomal system. For a thorough description of the events along the pathways and the therapeutic concepts illustrated in the figure, please refer to the text.

BMT/H SCT: Bone marrow transplantation/hematopoietic stem cell transplantation; ECM: Extracellular matrix; ERT: Enzyme replacement therapy.
evolutionary conserved pathway involved in the turnover of long-lived proteins and organelles and is essential for maintaining cellular energy and metabolic homeostasis [21-23]. There are three main modes of autophagy: macro-, micro- and chaperone-mediated autophagy; macroautophagy is characterized by a flat membrane cistern wrapping around cytoplasmic organelles and/or a portion of cytosol thereby forming a closed double-membrane bound vacuole, the autophagosome. The autophagosome finally fuses with lysosomes forming autophagolysosomes/autolysosomes, where the degradation and recycling of the engulfed macromolecules occur. Microautophagy is characterized by engulfment of cytosol by the lysosomes through invaginations of the lysosomal membrane. Besides the macromolecules, which are present in the engulfed cytosol, this process may also involve the uptake of organelles such as peroxisomes and mitochondria, with these particular autphagic processes being coined the terms pexo- and mitophagy, respectively [24,25]. Finally, chaperone-mediated transport of cytosolic proteins into the lysosomal lumen presents a more direct and selective form of autophagy. This pathway is dependent on the presence of the constitutively expressed member of the heat shock protein 70 (Hsp70) family, Hsc70 (HspA8), on both sides of the lysosomal membrane and its interaction with an isoform of the lysosomal membrane protein, LAMP-2, the protein that is defective in the LSD Danon disease [10,26].

1.2.4 Reformation of lysosomes and lysosomal secretion/exocytosis

After fusion of lysosomes with late endosomes or autophagosomes, the lysosomes are reformed from the resultant hybrid organelles through sequestration of membrane proteins and condensation of the luminal content [21]. The lysosomes, however, cannot be seen as the terminal point of the endocytic pathways as they are also able to form secretory lysosomes through fusion with secretory granules, a process that is Ca2+-dependent and was first recognized in secretory cells of hematopoietic origin [27,28]. However, evidence also exists for a Ca2+-regulated membrane-proximal lysosomal compartment responsible for exocytosis in non-secretory cells [29,30], a process which is dependent on the protein Rab27a, a member of the Rab protein family of small GTPases that have key regulatory roles in most membrane-transport steps including vesicle formation, motility, docking and fusion. At least 13 Rab proteins are utilized in the endocytic pathways in order to determine the fate of the various endocytosed molecules and their vesicles and alterations in the Rab GTPases or associated regulatory molecules give rise to a number of diseases ranging from bleeding and pigmentation disorders (Griscelli syndrome) through mental retardation and neuropathy (Charcot-Marie-Tooth disease) to kidney disease (tuberous sclerosis) and blindness (choroideremia) [11,31]. Ultimately, the reformation of lysosomes from autolysosomes (autophagosomes fused with lysosomes), endolysosomes (late endosome-lysosome fusion) and other lysosome-hybrid organelles completes each cycle of lysosomal degradation yielding functional lysosomes that are available for another round of macromolecular breakdown.

Importantly, the efficient processing of macromolecular substrates in the hybrid organelles are essential for lysosomal reformation, a process which does not occur de novo, but is the result of a reformation/budding from the hybrid organelles [32-34].

Defects in lysosome reformation which is thought to be required for the exocytosis of lysosomal cargo-containing membrane vesicles have also been shown to have pathological consequences as evidenced by studies in Niemann–Pick type C1 and C2 deficient cells (NPC1 and NPC2). These studies have revealed a significant difference in the lysosomal consequences of defects in the NPC1 and NPC2 proteins which otherwise share virtually indistinguishable clinical pathology in patients. For NPC1, one of the primary lysosomal consequences is a compromised ability to form the initial hybrid organelle, whereas in NPC2 deficient cells the impairment of lysosome reformation appears to be the primary cellular defect [7,35-38].

Interestingly, the impaired reformation of lysosomes could also constitute a more general molecular pathological feature of LSDs as secondary storage material in other LSDs, arising as a consequence of the primary genetic deficiency, could cause abnormalities in the necessary lipid dynamics involved in not only lysosomal reformation but also vesicle docking and fusion [39].

1.3 Lysosomal storage diseases

Individually, the LSDs are ultra-orphan diseases with prevalences ranging from 1/60,000 live births for Gaucher disease to 1/4.2 million live births for sialidosis. As a group however, the combined prevalence has been estimated to be ca. 1/7700 live births [40].

As delineated in the previous section, the interdynamics of the lysosomal system is a complex maze of a number of crucial events that all needs to function properly for the lysosomes to be the effective multifunctional organelles they are. The LSDs are excellent examples of the importance of these events as > 50 LSDs to this day have been characterized, ranging from primary defects in membrane proteins, transporters, fusion machinery and of course hydrolytic enzymes with the manifest cellular pathology associated with the diseases (lysosomal accumulation and storage) coining the name to these devastating diseases.

More than 50 LSDs are classified according to the major storage material accumulating, although their monogenetic origin does not always directly predict the affected substrates causing some discrepancies in understanding the molecular etiology of the diseases. In sphingolipidoses for example, the major storage materials are glycosphingolipids and immediate derivatives thereof, whereas for the neuronal ceroid lipofuscinoses, lipofuscins are the major storage materials accumulating, but on the background of a genetically and functionally
mixture of deficient proteases, peptidases, membrane proteins and other proteins with as yet unknown function.

The mechanisms by which the accumulated substrates impact cellular function and cause the pathological manifestations of the primary genetic defects are still not well understood, although several recent advances in the understanding of these processes are now shedding light into this dark(er) corner of human biology. These discoveries include mechanisms related to alterations of intracellular calcium homeostasis, impairment of autophagy, activation of signal transduction pathways by substrates and their derivatives, inflammation, altered intra- and limiting membrane properties and others [7,8,39,41-44].

Earlier, clinical management of LSDs was mainly confined to treatment of complications, although in some of the sphingolipidoses such as Gaucher and Fabry diseases attempts were made to improve the patients’ condition by transplantation of the major organs affected (liver and kidney, respectively), but these interventions did not alter the course of the diseases [45]. In mucopolysaccharidosis type I (Hurler syndrome, MPSIH), bone marrow transplantations has shown some benefit, however, provided the intervention is performed early enough [46]. The benefit of early intervention is a principle that holds not only for bone marrow transplantations but for all therapies applied to the LSDs, due to the irreversible nature of some of the pathological changes during the course of the diseases.

The breakthrough in the treatment of LSDs began modestly > 40 years ago when Neufeld and collaborators demonstrated the principle of metabolic complementation in cell cultures from patients with different LSDs [17,47]. Subsequent studies provided insight into the nature of the corrective factors (secreted lysosomal enzymes) and that these were endocytosed by binding to the M6PR [48]. These early studies showed that LSDs should be generally amenable to therapy relying on reconstitution of the deficient enzymes by exogenous administration of a functional version, a concept which is known as ERT. In some cell types, exogenous lysosomal enzymes are not recognized by the M6PR but rather by other receptor systems which bind, for example, terminal galactose (hepatocytes) or mannose residues (macrophages) [49]. That several receptor systems exist, needs careful consideration during the development of effective ERTs, but also holds opportunities for changing or modifying the targeting signals of a given enzyme in order to manipulate its pharmacodynamic properties. A classic example is the modification of glucosylceramidase that in order to be targeted to macrophages in Gaucher disease, has to be modified in order to expose mannose residues [49].

The success of ERT in Gaucher disease has made this approach the standard for treating lysosomal storage disorders (Table 1), although the first clinical trial of an ERT in 1973 (GM2-gangliosidosis) was not a clinical success although it did confirm the biochemical principle. In this trial, an infant was injected with hexosaminidase A that had been purified from human urine, resulting in a remarkable reduction of the storage substance in the circulation. However, the patient’s clinical condition remained unchanged [50]. The success of treating Gaucher and the apparent failure in treating GM2-gangliosidosis stresses the crucial point, that therapies should be developed that affect the primary sites of pathology. Unfortunately for the LSDs, the major organ involved in most of these diseases is the CNS which by no means is an easy organ to reach and even less so to rescue.

This realization has prompted the development of a number of therapies aimed not only at addressing the peripheral symptoms of the LSDs but importantly these novel concepts often aim at providing a clinical benefit in terms of manifestation of CNS-related symptoms, the holy grail of LSD therapy.

2. Medical need

The LSDs number over 50 diseases with a combined incidence of approx. 1/7700 and with only a very limited number having approved therapies available.

The clinical manifestations of these diseases are extremely variable ranging from severe debilitating, lethal diseases in early infancy, to attenuated presentations in late adulthood, often with no clear genotype/phenotype correlation as exemplified by Niemann–Pick disease type C in which the disease can vary from severe, lethal infantile disease to a more psychiatric symptom-driven disease that gradually manifest itself during the later decades [51].

The current standard of treatment has evolved from mainly supportive care and symptomatic treatment to the standard of today, ERT, that became available to patients with Gaucher disease two decades ago and now has also been developed and approved for other LSDs such as Fabry disease, MPS types I, II and VI and Pompe disease (Table 1). The efficacy of many of these therapies is limited however, due to the fact that the exogenously provided enzymes do not have effect on all aspects of the diseases. This is caused in part by the irreversibility of some aspects of these diseases but is also due to the enzyme formulations’ inherent inability to reach all major target organs in therapeutically efficacious amounts. Particularly the CNS, but also bone, cartilage, cardiovascular and renal systems are not necessarily efficiently targeted by enzyme replacement strategies due to the extremely selective permeability of the blood–brain barrier (BBB) and restricted receptor expression and lack of sufficient blood flow to support the needed doses for efficacy in other peripheral tissues and organs.

Furthermore, the formation of antibodies against the exogenously delivered enzyme may have a negative impact on efficacy as well as elicit unwanted infusion-related adverse events [52,53].

It comes as little surprise therefore, that there exists a substantial unmet need for the development of therapies addressing the limitations described above as well as providing alternative treatment regimens that eventually might even
supplement each other based on a rational understanding of their distinguishing mechanisms of action.

3. Existing and emerging therapies

The currently approved pharmacological therapies for LSDs are summarized in Table 1, while emerging therapies and therapeutic concepts are extensively covered in Tables 2 and 3. As can be readily seen, both established and emerging therapies are dominated by ERT or second-generation variants thereof.

The following sections provide a comprehensive overview of current and emerging therapies for the LSDs and will focus on the scientific and medical rationale for these therapeutic concepts.

3.1 Bone marrow transplantation/hematopoietic stem cell transplantation

Bone marrow and hematopoietic stem cell transplantations (BMT/HSCT) can trace the origin of their scientific rationale back to the same fundamental principle of metabolic cross-correction as the ERTs, that is, the ability of lysosomal enzymes to enter into a secretion-reuptake cycle. The first uses of transplantation approaches emerged in the 1980s and have seen its use in many of the LSDs including MPS types I, VI and VII, metachromatic leukodystrophy (MLD), alpha-mannosidosis, fucosidosis, Krabbe disease and type III Gaucher disease [54].

Despite a rather extensive use of BMT/HSCT in MPS type I and 1H (Hurler variant) and with some promising results, particularly in terms of reducing visceromegaly, cardiac function and airway obstruction [55], it is unfortunately still hard to conclude decisively on the status of this potentially effective therapy as most reports on BMT and HSCT are both anecdotal and/or only encompass a small number of patients.

However, with the advancement of methods for HSCT and with a more systematic approach in evaluating the therapy there is no scientific reason as to why BMT/HSCT should not be a both viable and a promising therapy for many of the LSDs, although several challenges such as the occurrence of variable musculoskeletal disease progression even after successful stem cell transplantation (SCT) in MPSIH patients have to be overcome [56,57].

3.2 Enzyme replacement therapies

As for bone marrow and hematopoietic SCTs the fundamental principle of ERT is the same: the substitution of the deficient enzyme by a functional version hereof.

Provided the enzyme can be manufactured and safely administered, the administration of the enzyme usually takes place through either weekly or biweekly infusions although more frequent administrations are also seen for some ERTs such as asfotase alfa in development for hypophosphatasia (Clinicaltrials identifier: NCT01176266).

The promise of most ERT's lies in their potential capacity to correct the pathology of non-neural tissue as the enzymes are incapable of traversing the BBB, although many peripheral tissues such as bone, cartilage, cardiovascular and renal systems are not easily reached due to the biology of the receptor systems needed for the endocytosis of the exogenously delivered enzymes.

Tables 1 and 2 summarize the ERTs that have been approved for marketing authorization and those that are in current development, respectively.

Table 1. Overview of marketed therapies available for LSDs.

<table>
<thead>
<tr>
<th>LSD</th>
<th>Drug</th>
<th>Company</th>
<th>Status</th>
<th>Mechanism</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaucher disease</td>
<td>Cerezyme® (miglucerase)</td>
<td>Genzyme</td>
<td>On market</td>
<td>ERT</td>
<td>Plant cell manufacture, only approved in the USA</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>VPRIV® (velaglucerase alfa)</td>
<td>Shire HGT</td>
<td>On market</td>
<td>ERT</td>
<td>Only for Gaucher type I patients for whom ERT is unsuitable</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>Elyso® (taliglucerase)</td>
<td>Protalix</td>
<td>On market</td>
<td>ERT</td>
<td>Not approved in the USA, approved in the EU, Asia and Canada</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Fabrazyme® (agalsidase beta)</td>
<td>Genzyme</td>
<td>On market</td>
<td>ERT</td>
<td>Not approved in the USA, approved in the EU, Canada, Switzerland, Brazil, Australia, Turkey and Israel</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Replagal® (agalsidase alfa)</td>
<td>Shire HGT</td>
<td>On market</td>
<td>ERT</td>
<td></td>
</tr>
<tr>
<td>Niemann-Pick type C disease</td>
<td>Zavesca (miglustat)</td>
<td>Actelion</td>
<td>On market</td>
<td>SRT</td>
<td></td>
</tr>
<tr>
<td>MPS type I</td>
<td>Aldurazyme® (laronidase)</td>
<td>Genzyme</td>
<td>On market</td>
<td>ERT</td>
<td></td>
</tr>
<tr>
<td>MPS type II</td>
<td>Elaprase® (idursulfase)</td>
<td>Shire HGT</td>
<td>On market</td>
<td>ERT</td>
<td></td>
</tr>
<tr>
<td>MPS type VI</td>
<td>Naglazyme® (galsulfase)</td>
<td>Biomarin</td>
<td>On market</td>
<td>ERT</td>
<td></td>
</tr>
<tr>
<td>Pompe disease</td>
<td>Myozyme® (agalglucosidase alfa)</td>
<td>Genzyme</td>
<td>On market</td>
<td>ERT</td>
<td></td>
</tr>
<tr>
<td>Cystinosis</td>
<td>Cystagon® (cysteamine)</td>
<td>Various</td>
<td>On market</td>
<td>Cysteamine suppl.</td>
<td>On market 1994</td>
</tr>
<tr>
<td>Cystinosis</td>
<td>Cystaran® (cysteamine)</td>
<td>Sigma-Tau Pharmaceuticals</td>
<td>Approved</td>
<td>Cysteamine suppl.</td>
<td>Cysteamine ophthalmic solution</td>
</tr>
</tbody>
</table>

ERT: Enzyme replacement therapy; LSD: Lysosomal storage disease; MPS: Mucopolysaccharidosis; SRT: Substrate reduction therapy.
## Table 2. Overview of ERTs and second-generation variants in commercial development for LSDs.

<table>
<thead>
<tr>
<th>LSD Drug candidate</th>
<th>Company</th>
<th>Status</th>
<th>Mechanism</th>
<th>Comments</th>
<th>CNS targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha-mannosidosis</td>
<td>Lamazym&lt;sup&gt;®&lt;/sup&gt; (alpha-o-mannosidase)</td>
<td>Zymenex</td>
<td>Phase II</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Migalastat + ERT</td>
<td>Amicus/GSK</td>
<td>Phase II</td>
<td>CCT/ERT</td>
<td>Combination approach</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>PRX-102 (α-galactosidase-A)</td>
<td>Protalix</td>
<td>Phase VII</td>
<td>ERT</td>
<td>Plant cell manufacture</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>JR-051 (α-galactosidase A)</td>
<td>JCR Pharmaceuticals/GSK</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>JR-101 (glucocerebrosidase)</td>
<td>JCR Pharmaceuticals/GSK</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>Oral glucocerebrosidase</td>
<td>Protalix</td>
<td>Preclinical</td>
<td>ERT</td>
<td>Oral ERT</td>
</tr>
<tr>
<td>Krabbe disease</td>
<td>Galaczym® (galactocerebrosidase)</td>
<td>Zymenex</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Late infantile NCL (Batten)</td>
<td>BMN-190 (TPP1)</td>
<td>Biomarin</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>MLD</td>
<td>Intrathecal HGT 1110 (aryl sulfatase A)</td>
<td>Shire HGT</td>
<td>Phase VII</td>
<td>ERT</td>
<td>Intrathecal delivery</td>
</tr>
<tr>
<td>MLD</td>
<td>AGT-183 (HIRmAb-aryl sulfatase A)</td>
<td>Armagen</td>
<td>Preclinical</td>
<td>Second-generation ERT</td>
<td>Insulin receptor antibody fused to aryl sulfatase A</td>
</tr>
<tr>
<td>MPS type I</td>
<td>AGT-181 (HIRmAb-iduronidase)</td>
<td>Armagen</td>
<td>Preclinical</td>
<td>Second-generation ERT</td>
<td>Insulin receptor antibody fused to iduronidase</td>
</tr>
<tr>
<td>MPS type II</td>
<td>Intrathecal Elaprase&lt;sup&gt;®&lt;/sup&gt; (iduronate-2-sulfatase)</td>
<td>Shire HGT</td>
<td>Phase VII</td>
<td>ERT</td>
<td>Intrathecal delivery</td>
</tr>
<tr>
<td>MPS type II</td>
<td>AGT-182 (HIRmAb-iduronate-2-sulfatase)</td>
<td>Armagen</td>
<td>Preclinical</td>
<td>Second-generation ERT</td>
<td>Insulin receptor antibody fused to iduronate-2-sulfatase</td>
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<td>MPS type III</td>
<td>JR-032 (iduronate-2-sulfatase)</td>
<td>JCR Pharmaceuticals/GSK</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>MPS type IIIA</td>
<td>Intrathecal HGT 1410 (heparan N-sulfatase)</td>
<td>Shire HGT</td>
<td>Phase VII</td>
<td>ERT</td>
<td>Intrathecal delivery</td>
</tr>
<tr>
<td>MPS type IIIB</td>
<td>Intrathecal HGT 3010</td>
<td>Shire HGT</td>
<td>Preclinical</td>
<td>ERT</td>
<td>Intrathecal delivery</td>
</tr>
<tr>
<td>MPS type IIIC</td>
<td>BSC-103 (α-N-acetyl-glucosaminidase)</td>
<td>Synageva</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>MPS type IIID</td>
<td>BMN-110 GALNS (N-acetylgalactosamine-6-sulfatase)</td>
<td>Biocytex</td>
<td>Phase III</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>MPS type VII</td>
<td>UX003 (β-glucuronidase)</td>
<td>Ultragenyx</td>
<td>Preclinical</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Niemann–Pick type B</td>
<td>ASM</td>
<td>Genzyme</td>
<td>Phase I</td>
<td>ERT</td>
<td>Carbohydrate remodeling to target CI-M6PR</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>Neo-rhGAA</td>
<td>Genzyme</td>
<td>Preclinical</td>
<td>Second-generation ERT</td>
<td>Fusion protein of IGF2 peptide and GAA</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>BMN-701 (IGF2-GAA)</td>
<td>Biocytex</td>
<td>Phase I</td>
<td>Second-generation ERT</td>
<td>Combination approach</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>Duvo-glustat + ERT</td>
<td>Amicus</td>
<td>Phase II</td>
<td>CECT/ERT</td>
<td>Engineered 95 kDa precursor to target CI-M6PR</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>OXY 2098-85 (modified GAA)</td>
<td>Oxyrase</td>
<td>Preclinical</td>
<td>Second-generation ERT</td>
<td>-</td>
</tr>
<tr>
<td>Wolman disease</td>
<td>Sebelipase alfa (lysosomal acid lipase)</td>
<td>Synageva</td>
<td>Phase II</td>
<td>ERT</td>
<td>-</td>
</tr>
<tr>
<td>Undisclosed LSDs</td>
<td>p97 (melanotransferrin)/ERT conjugate</td>
<td>Shire HGT/bioOasis</td>
<td>Discovery</td>
<td>Second-generation ERT</td>
<td>p97 to facilitate transport of ERT across BBB</td>
</tr>
<tr>
<td>Undisclosed LSDs</td>
<td>Angiopep-ERT conjugate</td>
<td>GSK/Angiochem</td>
<td>Discovery</td>
<td>Second-generation ERT</td>
<td>Angiopep targets LRP-1 to facilitate transport across BBB</td>
</tr>
</tbody>
</table>

ASM: Acid sphingomyelinasen; BBB: Blood–brain barrier; CCT: Chemical chaperone technology; CI-M6PR: Cation-independent mannose-6-phosphate receptor; CNS: Central nervous system; ERT: Enzyme replacement therapy; GAA: Acid alpha-glucosidase; HIRmAb: Monoclonal antibody to the human insulin receptor; IGF2: Insulin-like growth factor 2; LRP-1: Low-density lipoprotein receptor-related protein 1; LSD: Lysosomal storage disease; MLD: Metachromatic leukodystrophy; TPP1: Tripeptidyl peptidase-1.
T. Kirkegaard

Table 3. Overview of non-ERT emerging therapies in commercial development for LSDs.

<table>
<thead>
<tr>
<th>LSD</th>
<th>Drug candidate</th>
<th>Company</th>
<th>Status</th>
<th>Mechanism</th>
<th>Comments</th>
<th>CNS targeting</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple LSDs (sphingolipidoses amongst others (ao))</td>
<td>Orph-001 (Hsp70)</td>
<td>Orphazyme</td>
<td>Preclinical</td>
<td>MCT</td>
<td>Main receptor LRP-1, targets BBB</td>
<td>+</td>
</tr>
<tr>
<td>Multiple LSDs (orally available small molecules)</td>
<td>Orph-002</td>
<td>Orphazyme</td>
<td>Preclinical</td>
<td>MCT</td>
<td>Non-toxic, small molecule molecular chaperone inducers, BBB penetrating</td>
<td>+</td>
</tr>
<tr>
<td>Multiple LSDs (gangliosidoses)</td>
<td>Not disclosed</td>
<td>Zacharon Pharmaceuticals</td>
<td>Discovery</td>
<td>SRT</td>
<td>Ganglioside synthesis inhibition</td>
<td>+</td>
</tr>
<tr>
<td>Multiple LSDs (MPS types I, II and III)</td>
<td>Not disclosed</td>
<td>Zacharon Pharmaceuticals</td>
<td>Preclinical</td>
<td>SOT</td>
<td>Inhibition of 2-O sulfation of heparan sulfate</td>
<td>+</td>
</tr>
<tr>
<td>Gaucher disease</td>
<td>AT2101 + ERT, AT3375 + ERT</td>
<td>Amicus</td>
<td>Preclinical</td>
<td>CCT/ERT</td>
<td>Combination approach</td>
<td>-</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Migalast</td>
<td>Amicus/GSK</td>
<td>Phase III</td>
<td>CCT</td>
<td>Study did not meet primary end points</td>
<td>-</td>
</tr>
<tr>
<td>Fabry disease</td>
<td>Migalast + ERT</td>
<td>Amicus/GSK</td>
<td>Phase II</td>
<td>CCT/ERT</td>
<td>Combination approach</td>
<td>+</td>
</tr>
<tr>
<td>MPS type IIIa</td>
<td>SAF-301 (AAVrh.10-SGH- SUMF1)</td>
<td>Lysogene</td>
<td>Phase II/III</td>
<td>GT</td>
<td>Combination approach</td>
<td>-</td>
</tr>
<tr>
<td>MPS type IIb</td>
<td>Viral vector carrying α-N-acetylgalacosaminidase</td>
<td>UniQure</td>
<td>Phase II/III</td>
<td>GT</td>
<td>Combination approach</td>
<td>-</td>
</tr>
<tr>
<td>Niemann–Pick type C</td>
<td>HBP-cyclodextrin</td>
<td>NICHD</td>
<td>Phase I</td>
<td></td>
<td>Cholesterol sequestering agent, ICV administration</td>
<td>+</td>
</tr>
<tr>
<td>Pompe disease</td>
<td>Duvoglastat + ERT</td>
<td>Amicus/Raptor Pharmaceuticals</td>
<td>Phase II</td>
<td>CCT/ERT</td>
<td>Reformation approach</td>
<td>-</td>
</tr>
<tr>
<td>Cystinosis</td>
<td>RP103</td>
<td></td>
<td>Registration</td>
<td>Slow release cysteamine</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Combination approaches with ERT have been included.

BBB: Blood-brain barrier; CCT: Chemical chaperone technology; CNS: Central nervous system; ERT: Enzyme replacement therapy; GT: Gene therapy; Hsp70: Heat shock protein 70; ICV: Intracerebroventricular; LRP-1: Low-density lipoprotein receptor-related protein 1; LSD: Lysosomal storage disease; MCT: Molecular chaperone therapy; MPS: Mucopolysaccharidosis; NICHD: National Institute of Child Health and Human Development; SOT: Substrate optimization therapy; SRT: Substrate reduction therapy.

The first marketed ERT was developed for type I Gaucher disease, a sphingolipid storage disorder, characterized by splenomegaly, thrombocytopenia and anemia. The first clinical trial was performed by Brady and collaborators with an enzyme preparation purified from human placenta, treated with specific glycosidases to facilitate uptake by mannose receptors on the macrophages which is the main cell type involved in the disease [49]. Based on the positive data from this trial, the enzyme preparation (Ceredase®, Genzyme) was approved for patients with Gaucher disease and was some years later replaced by a recombinant form, imiglucerase (Cerezyme, Genzyme). A number of reports and publications have confirmed the long-lasting positive effect as well as the safety and tolerability of imiglucerase in patients suffering from type I Gaucher disease which has led to ERT becoming the standard of care for these patients [58,59] but does not have any influence on the CNS symptoms of the disease as seen in the neuropathic forms of the disease, not even at high doses [60].

The initial success of imiglucerase, has led to the development of ERT for other LSDs such as Fabry disease, Pompe disease and several of the MPS. In Fabry disease for example, two different α-galactosidase enzymes have been developed, but although Fabry disease is a glycosphingolipidosis just as Gaucher disease, its pathology is significantly different from that of Gaucher, giving rise to a number of challenges mainly associated with the proper engagement of the ERT with the target organs. In Fabry disease, kidney failure, cardiomyopathy and cerebrovascular events are the main complications which give rise to the morbidity and mortality associated with this disease while most clinical efficacy measurements have been based on plasma Gb3 levels (the main accumulating lipid) [61,62]. While plasma Gb3 levels are quite easily quantifiable and respond readily to intravenous injections of ERT, the main organs giving rise to disease symptomatology are neither as readily accessible to therapy nor as well investigated [61]. This inconsistency in addressing clinically relevant end points has made a clear conclusion on the benefits of ERT for Fabry disease difficult and the experience with ERT for Fabry disease has not been as satisfying as that in Gaucher disease, in which the pathological storage mainly involves a more easily targetable cell population. Also, recently the central role of Gb3 in Fabry disease and its relevance as a surrogate marker has been questioned as the lyso
Emerging therapies and therapeutic concepts for lysosomal storage diseases

derivative of Gb3 (globotriaosylsphingosine) may be a more pathologically relevant metabolite [63,64].

The findings in Fabry disease highlights the importance for a given therapy to reach the clinically relevant target organs in therapeutically efficacious doses, and the need to monitor this rigorously to aid conclusions on the effectiveness of a given therapy. It also points to the challenge that not only for Fabry disease, but for many of the LSDs, the current understanding of their molecular pathology is still far from complete.

If one turns to another group of LSDs, the MPS, the pattern observed for Fabry disease repeats itself. In MPS, there are 11 known genetic deficiencies all involving enzymes that are part of the catabolism of glycosaminoglycans (GAGs), giving rise to seven distinct MPS types. As with Fabry disease, the MPS are multisystemic diseases, affecting a multitude of cell types in a variety of tissues [65]. For MPS I, II and VI, ERT is now available [66-69], while first- or second-generation ERTs are also in development for several of the diseases (Table 2). For all of the approved ERTs, hepatosplenomegaly responds rapidly to the administration of intravenous enzyme with a concomitant reduction in urinary excretion of GAGs but as for Fabry disease, the main disease burden lies not within the easiest accessible organs, as the functional problems MPS patients suffer are rather due to skeletal dystosis and involvement of the soft tissues, heart and lungs. The clinical efficacy of ERT when measured in terms of joint mobility, vital capacity and walk tests, has not been nearly as clear as the effects observed on surrogate markers, and while the ERTs have evidently not been able to influence the CNS manifestations of the diseases, they have to some extent been able to alter the natural history of the disease and can in some cases improve the patient’s quality of life. As for the other LSDs, the earlier the intervention, the better.

The latest ERT marketed for a new LSD indication is alglucosidase alfa for the treatment of Pompe disease (glyco-gen storage disease type II, acid maltase deficiency), which is primarily affecting the muscle tissue, enabling a better ERT targeting of the clinically relevant tissue. For this disease, as for many of the LSDs, there is a broad spectrum of disease presentation with the most severe, infantile onset cases presenting in the first weeks after birth with both cardiovascular and skeletal myopathy while later onset cases usually see a sparing of the cardiovascular tissue but manifesting itself with a progressive proximal myopathy which can lead to respiratory failure, if involving the musculature of the diaphragm. The clinical efficacy of this ERT has been very encouraging with early intervention in the infantile cases being lifesaving, even in cases of advanced disease [70,71]. The efficacy of the ERT has furthermore been confirmed by several clinical trials in patients with different age of onset and disease severity and alglucosidase alfa has received approval for treatment of Pompe disease for all age groups [72].

It is evident that several factors define the clinical success of a given ERT: the primary challenge for any ERT lies in its clinical efficacy on the organ systems involved. As described, these systems vary from disease to disease but are often organs which are not readily accessible to ERT.

Although there are reports of enzymes successfully crossing the BBB in animal models of MLD and alpha-mannosidosis [73,74], clinical data for all commercially available ERTs have not shown any evidence of ERTs being able to penetrate the BBB and provide a therapeutic benefit to the patients. It is therefore of little surprise that all the second-generation ERTs (as well as many of the other emerging therapies) are aiming at either crossing the BBB and/or increasing the uptake of enzyme into the relevant peripheral tissue.

Of the latter there are currently several biologically similar approaches in development for the treatment of Pompe disease in which the primary tissue to target is the muscle tissue. Whether through carbohydrate remodeling of the enzyme itself, its conjugation to a IGF2-derived peptide tag or the engineering of a precursor form of the enzyme, all of these second-generation approaches aim at changing the biodistribution toward increased uptake in muscle tissue of the active enzyme by increasing its affinity to the CI-M6PR [75,77].

When it comes to targeting second-generation ERTs for transport across the BBB several approaches are in development. One approach is intrathecal delivery of standard ERT which is in clinical development for MPSII, MPSIIIA and MLD. Another and well-characterized alternative approach is the more physiological approach of utilizing endogenous receptor systems for enzyme transcytosis across the BBB, an approach that is considered to have several advantages compared with the rather invasive surgical procedure of intrathecal infusion of enzymes [78]. Although several receptor systems have been characterized that facilitate transport across the BBB, for the LSDs two main receptor systems are currently being targeted by various companies in the hope that these will provide access for the engineered enzymes to the neurons of the CNS in therapeutically relevant doses. These emerging approaches are targeting enzymes for the treatment of MPSI, MPSII, MLD and other LSDs to the CNS via either the insulin receptor or the probably best characterized blood–brain transcytosis receptor, LRP-1 (low-density lipoprotein receptor-related protein 1) (CD91) [79-84].

For the targeting of the insulin receptor, engineered versions of the enzyme are fused to the carboxyl terminus of the heavy chain of a chimeric monoclonal antibody (mAb) to the human insulin receptor (HIR). These HIRmAb–enzyme fusion proteins then cross the BBB via the endogenous insulin receptor and acts as a so-called molecular Trojan horse to ferry the enzyme into brain with approximately 2 – 3% of injected dose reaching the brain [80,85].

The LRP-1 and -2 have been exploited to target a large variety of drugs to the brain and LRP is the best characterized system for BBB penetration to date [78]. LRP-1 has a number of physiological ligands and this has formed the basis for two alternate fusion protein approaches, one relying on the conjugation of p97/melanotransferrin to the enzyme of interest, the other on an optimized peptide, a so-called angiopep,
increased affinity for the LRP-1 receptor [82,84]. Interestingly, the human molecular chaperone Hsp70 which is being developed for the treatment of a panel of LSDs and covered later in this review also utilize LRP-1 as one of its primary receptors [86].

Besides the above, a number of different targeting systems are being explored academically, such as intercellular adhesion molecule (ICAM) and apolipoprotein B (ApoB)-mediated carriers [87,88] but it is considered beyond this review to cover all early discovery pharmacology not in a company or company-related pipeline.

Based on the preclinical activity surrounding the second-generation ERTs, one can only hope that any of these approaches will prove successful, but a number of challenges are facing the development of these more extensively engineered enzymes: Although promising data have been generated in murine models of the disease for many of these compounds, only a marginal ratio of the total injected enzyme becomes available to the CNS and it remains to be seen if this amount of enzyme can confer the same therapeutic benefit in human subjects. This challenge unfortunately only becomes harder when one considers that chronic administration of these modified enzymes will be necessary for sustained effect in patients as this will almost certainly give rise to significant antibody responses, as has been seen for all ERTs, albeit to various degrees.

Also, the use of any receptor system begs the question as to what effect will be caused by the extra-physiological use of such a system to deliver drugs, that is, could any adverse signaling cascades be activated or will the natural receptor half-life, distribution and activity be compromised by this extra utilization. Also, does the receptor actively transport the drug across the BBB or is the drug just binding to the receptor and sequestered in the BBB endothelium? For LRP-1 for example, these considerations are not necessarily a major problem as the receptor is rather promiscuous with many ligands already using the receptor and as it also has one of the fastest transcytosis rates (transfer coefficient/Kin) of any BBB receptor system [89], adverse receptor signaling, saturation and re-distribution as well as drug sequestration in the endothelium should not pose a significant risk for this system although this of course remains to be tested clinically.

Given all the challenges that remain to be faced by the second-generation ERTs, one should bear in mind however, that significant clinical benefit has been achieved for other diseases in which receptor systems have been exploited, for example, in the case for dopamine/L-Dopa for Parkinson’s disease patients, in which the large neutral amino acid carrier has been used to deliver L-Dopa, the metabolic precursor of dopamine, to the brain resulting in a clear clinical benefit as dopamine in itself is not able to cross the BBB.

### 3.3 Substrate reduction therapies

Whereas ERTs focus on increasing the catabolism of build-up substrate, the principle in substrate reduction therapy (SRT) is to limit the production of substrate to the catabolically compromised lysosomes. The first demonstration of this principle was done by Platt et al. in 1994 with the imino sugar N-butyldeoxynojirimycin (NB-DNJ, miglustat, Zavesca®) which has the ability to inhibit the enzymatic activity of ceramide glucosyltransferase (glucosylceramide synthase) which synthesizes glucosylceramide, the precursor of several glycosphingolipids such as the globo- and gangliosides, and which is the main accumulating lipid in Gaucher disease [90]. Miglustat was subsequently tested in a clinical trial with 28 Gaucher disease patients, who for several reasons did not receive ERT and on basis of this trial miglustat gained marketing approval in Europe and the USA for the treatment of adult patients with mild to moderate type 1 Gaucher disease for whom ERT is not a therapeutic option [91].

An SRT based on the inhibition of ceramide glucosyltransferase holds the potential of being a therapy for all LSDs with glycosphingolipid storage and since miglustat crosses the BBB, this therapy has been evaluated for a number of sphingolipidoses with prominent neurodegeneration such as Tay–Sachs disease, type 3 Gaucher disease, MPSIII, juvenile GM2-gangliosidosis and Niemann–Pick type C disease [92,94]. Except for Niemann–Pick type C disease, none of these trials have shown improvement in the miglustat-treated patients although some of these data should be handled with care as the studies were done on very limited numbers of patients. For Niemann–Pick type C, miglustat received marketing approval in Europe in 2009 based on a clinical trial in patients aged 12 or older, which demonstrated that treatment with miglustat improved eye movement velocity and swallowing capacity [95].

Being a small molecule sugar analog, the side-effect profile of miglustat is significantly different from the side-effect profiles associated with ERTs with miglustat having a broader array of side effects including gastrointestinal symptoms, particularly diarrhea.

A conceptually similar, but chemically different, approach for SRT centered on ceramide-based inhibitors of ceramide glucosyltransferase provides a novel alternative to the imino sugar-based SRT. Based on this approach, a new inhibitor of ceramide glucosyltransferase, eliglustat tartrate (Genz-112638) is currently in development for Gaucher disease type I, and has shown promising results in an open-label Phase II trial, combining a higher specificity for ceramide glucosyltransferase with a more beneficial side-effect profile compared with miglustat and having a clear effect on several disease parameters [97].

For the MPS, the accumulation of GAGs can be inhibited by genistein, an isoflavone extract from soybeans being explored academically. The effect of genistein on urinary GAG excretion, hair morphology and behavior has been tested in an open-label study of 10 patients suffering from either MPSIIIA or IIIB and a 2-year follow-up including eight patients assessing the cognitive function and general status was recently published. Albeit consisting of a small number...
of patients, after 1 year of oral administration of a genistein-rich soy isoflavone extract (5 mg/kg/day), a statistically significant improvement was observed with a larger variance in efficacy being apparent after 2 years [98].

### 3.4 Chaperone technologies

As most LSDs are characterized by significantly reduced enzyme activity due to missense mutations rather than a complete loss of function, the LSDs have long been thought amenable to chaperoning by chemical substrate mimics targeting the active site of the relevant enzyme for increased stability/folding.

A more recent approach relies on utilizing the already existing molecular chaperone machinery available in the cells in order to avoid the inherently counterproductive mechanism of enzyme inhibition associated with chemical chaperone therapies.

The advantages to both approaches compared with ERT include better distribution profiles including CNS availability as well as easier drug administration as the small molecule approaches for both concepts offer the potential of oral administration rather than the more patient-demanding infusions of ERT.

#### 3.4.1 Molecular chaperone technologies

There are currently two approaches in development for utilizing the naturally occurring molecular chaperone machinery, both exploiting the recently discovered mechanism for how the archetypical molecular chaperone Hsp70 aside from its well-characterized cytoprotective effects also enhances cell survival and functionality through a direct lysosomal action [43]. One approach relies on the receptor-mediated endocytic uptake of a recombinant version of Hsp70 whereas the second approach relies on utilizing small molecules capable of enhancing the endogenous production of heat shock proteins, here amongst Hsp70. Hsp70 is an evolutionarily highly conserved molecular chaperone which has been shown to promote the survival of stressed cells by inhibiting lysosomal membrane permeabilization [99-101], a hallmark of stress-induced cell death [6,102]. Recently, Kirkegaard et al. described how Hsp70 stabilizes lysosomes by binding to the endolysosomal anionic phospholipid bis(monoacylgllycerol)phosphate (BMP), an essential co-factor for lysosomal sphingolipid metabolism hereby facilitating the BMP binding and increased activity of acid sphingomyelinase (ASM), the enzyme compromised in Niemann–Pick diseases. Notably, the reduced ASM activity in cells from patients with Niemann–Pick disease A and B was shown to associate with a marked decrease in lysosomal stability, and this phenotype as well as the pathological accumulation of unstable lysosomes could be effectively corrected by treatment with recombinant Hsp70. The mechanism of action of Hsp70 entails the prospect of using the protein for the treatment of several LSDs, most notably the sphingolipidoses involving enzymes that are dependent on interaction with BMP [103] and it is currently in preclinical development for a number of these diseases.

The approach to utilize small molecules to increase the expression of heat shock proteins during pathological stress conditions and harness this response for therapeutic use, stems from the ability of these molecules to stabilize the transcription factor for the heat shock proteins, heat shock factor-1 (HSF-1) [104]. Interestingly, this emerging approach for LSDs is also in development for a number of neurodegenerative conditions, including amyotrophic lateral sclerosis and has a well-described safety record with very limited side effects, which could possibly accelerate the development of this therapeutic concept for LSDs [105].

#### 3.4.2 Chemical chaperone technologies

Contrary to the molecular chaperone approach which utilizes the potentiating effects of endogenous cellular chaperones, chemical chaperone technologies rely on using competitive inhibitors of lysosomal enzymes at subinhibitory concentrations in order to facilitate the transition of poorly folded lysosomal enzymes otherwise caught in the ER/proteasomal degradation machinery to the lysosomes as first described by Fan et al in 1999 [106]. On maturation and entry in the lysosomes, the concept demands that the kinetics of the enzyme/inhibitor interaction are shifted due to for example, the reduced pH of the lysosomes, facilitating the dissociation of the inhibitor and the enzyme, thus finally leaving a larger fraction of the functionally compromised enzyme available for increased substrate degradation in the lysosome [106,107].

A number of molecules are in development for LSDs based on this approach, including combination efforts with ERTs. The most advanced program for a LSD utilizing a chemical chaperone as stand-alone therapy is deoxygalactononjirimycin (DGJ; migalastat hydrochloride) which is currently in Phase III for Fabry disease. Despite a considerable power in this study as 67 patients diagnosed with Fabry disease with genetic mutations amenable to chaperone monotherapy were enrolled, the study recently reported an initial negative outcome, as it did not meet any of its primary end points during its first phase [108].

Although not in formal development programs for LSDs, the Food and Drug Administration (FDA)-approved drugs pyrimethamine and ambroxol have been identified as possible chemical chaperones for hexosaminidase A and ceramide glucosyltransferase and both have been tested in cells from patients suffering from late-onset forms of GM2-gangliosidosis (Tay–Sachs and Sandhoff disease) and Gaucher disease [109,110]. Recently, data from a small-scale open-label Phase I/II clinical study of the tolerability and efficacy of pyrimethamine in Sandhoff disease patients have been reported [111]. A significant side-effect profile was observed at doses of 75 mg pyrimethamine daily, while variable enzyme activity enhancement was seen at 50 mg/day. Although the design of the study does not allow for proper conclusions, the significant side-effect profile characterized by neurological side effects such as ataxia and
incoordination experienced in all subjects of the study, and the very narrow window to the dose conferring a seemingly increased enzymatic activity presents significant challenges for the further development of this compound.

Combination therapies of chemical chaperones and ERTs are being pursued for Gaucher, Fabry and Pompe diseases and are currently in preclinical (Gaucher) and Phase II (Fabry and Pompe) stages of development. Given the recent development challenges of the chemical chaperones (duvoglustat and migalastat) for Pompe and Fabry disease, respectively, the evaluation of these inhibitors in ERT combination studies for Pompe and Fabry diseases will be interesting.

3.5 Substrate optimization
The mechanism of action of current therapies targeting the substrates accumulates in LSDs such as miglustat and eliglustat with the focus on the inhibition of enzymes necessary for substrate biosynthesis and as such this approach entails the risk of reducing the substrate to a level where its normal functions are compromised. As an alternative to this, a concept has been described in which small molecules are used, not to prevent synthesis of substrates, but rather to modify their biosynthesis in order to change the structure of the substrate, which then no longer is dependent on the deficient enzyme for degradation but can be degraded by alternative enzymes with normal function [112]. This strategy has been termed substrate optimization therapy and is currently in development for MPS types I, II and III in which the targets for the substrate optimization are glucosaminoglycans (GAGs). By compound library screening, 15 inhibitors of GAG synthesis were identified that can be categorized into N-, 2-O-, or 6-O sulfation inhibitors. A 2-O sulfation inhibitor, ZIP2345, could reduce the levels of 2-O sulfation with a compensatory increase in 6-O sulfation of heparan sulfate with the modified heparan sulfate being more amenable to degradation in vitro in fibroblasts from MPS type II patients (iduronate sulfatase deficiency) [113]. Albeit still early stage, the technology has the potential of being able to target more than one MPS with the same compound as well as being able to cross the BBB and other organs that have proven hard to reach for ERTs.

3.6 Gene therapy
A number of gene therapeutic approaches using retro-, lentiviral- or adeno-associated viral vectors have been evaluated pharmaceutically in a comprehensive array of animal models of LSDs and the general conclusion is that genetic therapy can show clear disease-modifying capacity in vitro [114]. The main challenges that remain to be overcome are the transfer of these findings to larger brains (the human brain volume is ca. 2000 times that of the mice, making efficient pan-cerebral delivery a challenge even with intracranial injections), the possible immunogenic responses to the viral vectors carrying the gene of interest as well as the potential need for immune suppression for the patients who are complete null for the enzyme. Despite these challenges, a number of clinical trials have been initiated for the LSDs, but as is the case for many trials within the LSD field the trials are featuring small number of patients and solid conclusions are hard to make as only a small number of studies have been completed. Reports on completed studies of gene therapy in Gaucher disease and MPS type II using retroviral vectors showed low expression of the gene product and no improvement in disease pathology [115,116]. However, these were the first two clinical trials of gene therapy in LSDs and since their initiation more than a decade ago there has been a marked development and improvement of vector design and delivery which will hopefully lead to improved results. Although a recently completed study in Batten disease involving direct injection of a recombinant adeno-associated viral (rAAV) serotype 2-based vector into the CNS of affected children indicated that progression of disease might have been slowed, a clear therapeutic benefit was not established and significant serious adverse events were also encountered, the causes of which could not be identified [117]. However, as for the retroviruses, rAAV-based gene therapy has also evolved substantially since the initiation of this study and several studies are underway utilizing other rAAV serotype vectors in for example, Pompe disease [118] and MPS types IIIA and IIIB, the latter two being researched and developed in commercial settings. The ongoing Pompe disease study uses a rAAV serotype 1 (rAAV1) vector encoding acid-α-glucosidase, and recently reported preliminary findings [118]. No adverse events or systemic toxicities related to vector administration were reported and significant elevation in respiratory parameters was noted for the first cohort of patients on the lower dose of treatment. These findings are encouraging for the development of gene therapies for LSDs, but the hope that all pathological components of a given LSD can be corrected by a systemic delivery of a single vector is probably too optimistic as the complex nature of these devastating diseases makes even this approach extremely difficult. A number of factors such as the timing of gene transfer in relation to diagnosis and symptom onset/aggravation, the variable levels of gene product needed to efficaciously treat various organ pathologies and the possible immune consequences related to administration procedures, choice of vector and naivety to gene therapy product will all have to be addressed in order to bring about the full potential of this therapeutic approach.

On top of these scientific and development challenges, challenges regarding regulatory considerations and commercial feasibility of developing gene therapies are also considerable. Very encouragingly however, for gene therapy as a future therapy for a number of genetic diseases, not only the LSDs, the first European regulatory approval of a gene therapy was recently announced, with this therapy also being based on an rAAV1 vector, AAV1-LPLS447X (aliopgene tiparvovec, Glybera®) targeting lipoprotein lipase deficiency, an ultra-rare genetic disease [119].
3.7 Other therapies and emerging approaches

The LSD cystinosis involves lysosomal storage of the amino acid cystine in all organs and tissues due to a defect in the lysosomal membrane transport protein, cystinosin, and was the first LSD recognized to be due to defective lysosomal membrane transport, and thus serves as a prototype for a small group of lysosomal transport disorders. In 1994, a novel therapy was approved based on depleting cystine in the form of orally administered cysteamine bitartrate (Cystagon®), which has revolutionized the management and prognosis of nephropathic cystinosis [120]. On administration, cysteamine enters the lysosomes and reacts with cystine, forming the mixed disulfide of half cystine (cysteine) and cysteamine. This complex can then exit the lysosomes via the transport system for cationic amino acids [121]. The efficacy of cysteamine has been validated in a number of studies and cysteamine therapy should be considered for all affected individuals, regardless of age and transplantation status [122].

The side-effect profile of cysteamine includes unpleasant taste, nausea and other digestive issues with the most common side effect being nausea that can be alleviated with antiemetics in the early stages of therapy initiation [122]. A different route of administration targets photophobia associated with the disease as topical cysteamine eye drops, administered every 1 – 2 h, dissolve corneal crystals and ameliorate this part of the pathology within a few weeks [123].

As nonsense mutations have been identified in a number of LSDs, leading to premature translation termination and the synthesis of truncated protein as in the case of the Arg220X mutation in Fabry disease, and as there exist evidence that small molecule drugs such as gentamicin can induce the readthrough of such premature stop codons, bringing about increases in otherwise null enzymatic activity, the concept of stop-codon readthrough has been explored in the severe form of MPS type I (Hurler disease). In cell cultures of patient fibroblasts carrying different nonsense mutations, gentamicin treatment increased the α-L-iduronidase activity in all cell lines tested except one providing an initial in vitro proof-of-concept for this approach [124]. Further development of amino-glycoside analogs exhibiting reduced cell toxicity and superior readthrough efficiency compared with gentamicin might hold hopes for an even better therapeutic future for this emerging concept [125]. In addition to the aminoglycosides, a novel chemical compound was recently identified, which selectively induces ribosomal readthrough of premature, but not normal termination codons [126]. This compound, PCT124 (ataluren), has entered clinical trials and might hold great potential for genetic disorders such as cystic fibrosis, for which no other therapeutic options are available [127].

As exemplified with the use of cysteamine for cystinosin, the reduction of storage material might not only be achieved by enhancement of enzymatic activity or inhibition of substrate synthesis as covered in the previous sections. Accumulating substrates might also be eliminated by substances such as 2-hydroxy-propyl-β-cyclodextrin, which is capable of binding unesterified cholesterol and other hydrophobic molecules. As unesterified cholesterol is one of the major storage compounds in Niemann-Pick type C disease, the compound has been tested in both the murine and feline model of the disease. Encouraging data from the most commonly used mouse model of the disease (the NPC<sub>csh</sub> model) led to the FDA approval of a compassionate use trial of cyclodextrin in a small number of patients suffering from advanced Niemann-Pick type C disease [128-130]. As cyclodextrins have been widely used as formulation vehicles to increase the amount of drug, including hormones and vitamins, which can be solubilized in aqueous vehicles [131], its use and toxicological profile has been extensively studied in rodents, dogs and monkeys where it is well tolerated at low doses [131,132]. However, daily i.v. administration of greater than 200 mg/kg caused reduced body weight, foamy macrophage infiltration of the lungs, elevations in hepatic enzymes, increased Kupffer cells in the liver and renal cortical tubular vacuolization in rodents [131,133,134].

Doses used to reach therapeutic effect in the murine model of Niemann-Pick type C are several-fold higher than the doses at which no adverse events are seen (4000 mg/kg used in the NPC mouse models) and apart from the available toxicological data in healthy animals increasing data from animal models of LSDs strongly suggest that the use of this compound for any LSD should be carefully evaluated as a number of side effects have been seen on treatment including hearing loss and increased cholesterol burden and macrophage infiltration of the lungs [128,135-137]. Furthermore, as cyclodextrin does not cross the BBB [138] and as its use in other murine models of cholesterol-storing LSDs such as GM1-gangliosidosis and MPSIIIA had no effect on storage [130], it is clear that the mechanism of action is not fully understood. Addressing these challenges will hopefully aid the development and future therapeutic approaches relying on this class of compounds.

Based on the complex pathology of the LSDs and the various cellular and biological organelles and processes involved, a number of experimental strategies, some including commercially available compounds, are being researched for their use as disease modifiers. These approaches include calcium modulation, enhancing exocytosis, regulation of proteostasis, modulation of autophagy and the use of non-steroidal anti-inflammatory drugs [42,139-145]. While most of these approaches are at an early stage and still have a long way ahead to the clinic, it is clear that a multifaceted approach is most likely needed to address the complex pathology of LSDs. No doubt, as the understanding of disease pathology advance, additional creative approaches to treatment will emerge and undergo similar early development with the hope that any of these approaches ultimately lead to clinical benefit for the patients.

4. Competitive environment

Table 1 summarizes the current status of the competitive environment for LSD therapies with market approval,
while Tables 2 and 3 summarize the various emerging therapies and concepts and provide a thorough overview of the status of the programs in primarily commercial development pipelines for the LSDs.

The emerging concepts being explored for LSDs with high unmet clinical needs or unaddressed pathology such as CNS deterioration is an exciting field which holds a number of promises for complimentary mechanisms of action offering a potentially larger arsenal of drugs for future therapy but in addition hereto, a number of primarily ERT projects are also being developed for LSDs with established and effective therapy as for example, type 1 Gaucher disease.

For type 1 Gaucher disease, three therapies are already commercially available (two ERTs (imiglucerase and velaglucerase alfa) and one SRT (miglustat)), with two ERT programs, a CCT/ERT combination program and an SRT also currently in development. The two ERT programs are based on alternative manufacturing systems and whether the products of these programs have significant differentiating factors with therapeutic relevance to the established therapies will be interesting to follow. Interestingly, one of these products, taliglucerase (Elelyso®, Protalix/Pfizer), was recently approved by the FDA (1 May 2012), but was rejected marketing approval by the EU commission as velaglucerase alfa (VPRIV®) has 10 years marketing exclusivity under the orphan drug framework.

5. Expert opinion

The current state of the LSD field is both complex and encouraging. Complex as the understanding of the underlying molecular pathology of the vast heterogeneity in clinical presentation is still limited and constantly evolves. Encouraging as novel therapeutic approaches evolve from these discoveries, carrying with them the hope that they may eventually impact the course of these devastating diseases.

Among this multitude of approaches, the ERTs still reign supreme in number of products commercially available, as well as number of programs in the development pipelines of pharmaceutical companies, although a number of alternative approaches are emerging.

Gaucher disease was the first LSD for which ERT became available and the impact made by this approach not only clinically but also commercially subsequently prompted the development of ERTs for a number of other LSDs. Recently, a 10-year follow-up study of Gaucher disease patients treated with ERT reported the lasting impact on patients health by treatment with imiglucerase but the same degree of success has unfortunately not been achieved with all ERTs although early intervention in for example, infantile Pompe disease has also been a marked success as this has proved life-saving [59,70]. Importantly, lessons learned from the use of ERTs in the clinic have clarified the challenges still facing ERTs and these are by no means trivial; adverse immunological reactions to infusion of enzyme are common and many sites of pathology are not effectively treated by intravenous administration of enzyme as these sites are not easily reached by the infused enzyme. In addition, some manifestations of disease has proven very hard to alleviate such as bone disease in Gaucher disease and the MPS, renal complications in Fabry disease and the degeneration of the CNS observed in many LSDs. All of these sites provide therapeutic targets which have yet to be efficiently reached by an ERT or therapeutic variant thereof.

A major therapeutic advancement will therefore be molecules which can reach these sites of pathology and a number of the emerging therapies are indeed aiming at exactly this, with therapies being able to cross the BBB being a particular focus for many of these development efforts. Until now only one drug approved for an LSD has shown indications that it might affect CNS complications. The orally administered SRT, miglustat, was first approved for the treatment of adult patients with mild to moderate type 1 Gaucher disease for whom ERT is not a therapeutic option [91] and has since been approved in the EU and other countries for the treatment of Niemann–Pick type C based on a clinical trial, which demonstrated that treatment with miglustat improved eye movement velocity and swallowing capacity indicating an effect on CNS pathology [95]. The challenges facing SRTs are based on their inherently unspecific mechanism of action as the inhibition of ceramide glucosyltransferase (an early core component in the glycosphingolipid synthesis pathway) by virtue of the sequential synthesis steps in this pathway unavoidably affects the synthesis and equilibrium of all downstream derivatives. Furthermore, miglustat is known to inhibit several glycosidases, including a-glucosidase I and II as well as sucrase and maltase, which might also explain parts of its side-effect profile [146]. This inherent non-specificity raises concerns about long-term toxicities or adverse events for any SRT but to date the side effects such as diarrhea have been controllable, although there are still concerns regarding the tremors and paresthesias that develop in some treated patients [91]. A novel SRT in development, eliglustat tartrate, has shown promising data including a more benign side-effect profile, probably owing to its higher selectivity, but unfortunately this agent is a target for P-glycoprotein meaning that it is efficiently pumped out of the brain and hence will most likely not have an impact on CNS disease in patients [97,147,148]. Nevertheless, the higher specificity and milder adverse events indicate that this compound hold great potential for being an efficacious SRT for the treatment of peripheral disease in Gaucher disease and other sphingolipidoses, and the promise of SRT still spurs design of new agents combining increased specificity and brain penetrating properties, providing a continued flow of potentially beneficial drugs for a large subset of the LSDs [148].

Gene therapy strategies are a possibly very important intervention which has seen a breakthrough with the EU approval of the first gene therapy (Glybera). This approach might offer an alternative to existing therapies as might oral approaches
and approaches with mechanisms of action across diseases such as the SRT and molecular chaperone therapies.

In case of chaperone approaches to treating LSDs, two intriguing concepts with the potential of addressing several LSDs including diseases with CNS involvement are in development. These are approaches that employ either endogenous molecular or chemical chaperones, respectively. The approaches are inherently dissimilar as the molecular chaperone therapy approach utilizes the endogenous chaperone machinery to not only enhance the activity of compromised enzymes but also provide the dysfunctional lysosomes and cells with the survival promoting benefits of the heat shock proteins [43,103].

Almost counterintuitively, chemical chaperone technologies use competitive inhibitors to enhance residual enzyme activity, with the technology having a plausible theoretical basis in the conformational memory of proteins. CCT uses competitive inhibitors of lysosomal enzymes at subinhibitory concentrations in order to facilitate the transition of poorly folded lysosomal enzymes otherwise caught in the ER/proteasomal degradation machinery to the lysosomes [106]. The technology has seen widespread development with a number of programs in current clinical trials, but recent clinical data have highlighted the difficulties in using inhibitors in already severely compromised enzymatic systems. The most recent setback being reported only recently as initial data from a Phase III randomized, placebo-controlled study of migalastat in Fabry disease including 67 patients showed that the study did not meet its primary end points [108].

A major aid to any clinical trial and a significant advancement to the understanding of LSDs will be the development of tests/biomarkers that accurately predict the disease outcomes and aggressiveness. Tools for better and early diagnosis are also needed as the often irreversible degeneration and pathology observed for many of these diseases prompt an early and efficient intervention in order to have the highest likelihood of significantly improving the clinical outcome. The development of these tools will be important not just for the understanding of the diseases and their progression but will hopefully allow for better clinical trial designs and a better definition of subpopulations of patients that will have better or poorer responses to therapies.

As early intervention is generally considered a prerequisite for the prevention of irreversible complications in any LSD, the development of suitable biomarkers is of significant importance as their absence makes it difficult to support presymptomatic pharmacological therapy without the capacity to monitor consequences of the intervention. Of course, early lifesaving interventions are completely unethical to withhold in for example, infantile Pompe, Krabbe and Niemann–Pick type A disease, but when should one intervene in more gradually developing diseases? As the current therapies are not benign and have not only medical but also social and economical implications for the patient in terms of insurability and employability, the initiation of therapy needs very careful consideration which will also be the case for the therapies in development. However, progress in biomarker characterization is being made for a number of diseases such as Gaucher disease, Fabry disease, the MPS and Niemann–Pick type C disease [149–151].

No doubt the number of emerging therapies for LSDs is encouraging, with a lot of exciting biological mechanisms being explored as potential drug targets. This is however, also necessary as the complex nature of the diseases almost certainly demands a combined effort targeting not only general or specific pathology but rather aims at a shot-gun approach for treatment utilizing a battery of therapeutic modalities to deal with the intricate molecular pathology underlying these devastating diseases.

Importantly, the significant cost of therapy for these very rare diseases has to be considered not only at an individual level, but also by physicians and society at large as we seek to improve the life of patients with LSDs. This consideration becomes of particular relevance in the case of combination therapies, which will most likely be the future way to improve the life of patients who by odd chance have developed such devastating diseases.

**Declaration of interest**

The author is employed by and holds shares in Orphazyme ApS, which develops therapies for lysosomal storage diseases.
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