Emptying the stores: lysosomal diseases and therapeutic strategies

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Abstract | Lysosomal storage disorders (LSDs) — designated as 'orphan' diseases — are inborn errors of metabolism caused by defects in genes that encode proteins involved in various aspects of lysosomal homeostasis. For many years, LSDs were viewed as unattractive targets for the development of therapies owing to their low prevalence. However, the development and success of the first commercial biologic therapy for an LSD — enzyme replacement therapy for type 1 Gaucher disease — coupled with regulatory incentives rapidly catalysed commercial interest in therapeutically targeting LSDs. Despite ongoing challenges, various therapeutic strategies for LSDs now exist, with many agents approved, undergoing clinical trials or in preclinical development.

Lysosomal storage disorders
(LSDs). Disorders in which macromolecules build up in lysosomes, leading to so-called 'storage' as a result of an inherited mutation in a gene involved in lysosomal function.

Multimorbidity
Individuals with multiple, typically chronic, clinical conditions.

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Lysosomal storage disorders (LSDs) are a family of over 70 rare monogenic diseases that typically present in infancy or childhood and collectively affect 1 in 5,000 live births[1]. However, adult-onset forms also occur, and as these are frequently misdiagnosed, they are likely to be more prevalent than currently believed[2–4]. The vast majority of LSDs share the common cellular feature of an expanded lysosomal system, caused by the accumulation of a variety of cellular macromolecules (storage). The storage materials differ biochemically in each disease, reflecting the nature of the primary genetic defect[5]. Most of the causative genes encode lysosomal enzymes or proteins involved in lysosomal enzyme modification or transport, but they can also encode lysosomal membrane proteins[6]. When a lysosomal enzyme is deficient, its substrate or substrates are stored. When a membrane protein is defective, the pattern of storage can be complex, depending on the function of the protein in question. The genetics and biochemical nature of the storage substrates for most LSDs are well defined; however, we still have incomplete knowledge of how lysosomal dysfunction triggers the complex cellular pathogenic cascades that occur in LSDs, causing cell dysfunction and ultimately cell death[7].

Approximately 70% of LSDs present as progressive neurodegenerative diseases, highlighting how vulnerable the central nervous system (CNS) is to lysosomal dysfunction[5]. In addition, peripheral organs and tissues are also often affected in these diseases, and the majority are therefore chronic, multimorbidity diseases. This has important implications for the development of effective therapies, as multiple compartments of the body may require correction or effective treatment.

The availability of animal models of LSDs in multiple species (typically rodents, companion animals and livestock species) has supported the study of pathogenesis and greatly facilitated translational activity[8]. Rare and ultra-rare diseases such as LSDs, with complex pathophysiology often involving the brain, have not historically been the focus of pharmaceutical industry interest. However, LSDs are currently a burgeoning translational field with multiple approved products in routine clinical use and intense academic and commercial activity promoting the innovation of new therapeutic approaches at a remarkable rate[9]. The trigger for the translational activity in this field was the pioneering academic and commercial effort to develop the first biologic therapy for an LSD, an enzyme replacement therapy (ERT) for type 1 Gaucher disease[10]. In addition, an innovative regulatory framework to support and promote the commercial development of therapies for orphan diseases was formed (BOX 1).

The development of biologics and more recently small-molecule drugs for LSDs has made it more important than ever that patients with these diseases are correctly diagnosed and treated as early as possible to maximize therapeutic benefit. Newborn screening is an expanding area that aims to identify patients at birth and instigate treatment rapidly, should a therapy be available[11,12] (FIG. 1). Early diagnosis of the first affected individual in a family also provides the parents reproductive options to prevent the birth of other affected individuals in the future. The ethical dilemmas of newborn screens are complex, and how mutations of unknown significance are handled remains a serious concern, as there is a considerable risk of branding a healthy infant with an LSD diagnosis that may never manifest clinically in the individual's lifetime[13] (FIG. 1).

Biologic therapies
Treatments derived from living organisms to treat a disease. In the field of lysosomal storage disorders, enzyme replacement therapy, cell transplantation and gene therapy are all biologic therapies.

Lysosome
An acidic organelle involved in macromolecule catabolism and recycling but also plays a role in nutrient sensing and calcium signalling.

This Review provides an overview of LSDs and assesses the challenges associated with their diagnosis, drug development and treatment. We are now in an exciting translational era where the biologic therapies that have been the cornerstone of treatment to date are being complemented by a diverse range of small molecules and nucleic acid-based therapies. Therapeutic approaches that either have been approved or are in clinical trials or those for which advanced preclinical proof of concept has been demonstrated are discussed.

The lysosome

The lysosome is an acidic organelle that serves as the major catabolic and recycling centre of nucleated cells[14]. The biogenesis of lysosomes is tightly regulated, along with autophagic pathways, by the coordinated lysosomal expression and regulation (CLEAR) gene network[14–16], which is under the control of the master transcription factor EB (TFEB)[17–19] in cooperation with TFE3 (REF. 20). The wider lysosomal system is now appreciated as playing a central role in nutrient sensing, general energy metabolism and the response of the body to exercise[19,20] as well as regulating aspects of cholesterol homeostasis[21].

The lysosome contains numerous acid hydrolases required for macromolecule catabolism. The limiting membrane of the lysosome is populated with over 300 membrane proteins[22,23], many of which are known to be involved in lysosomal function. This includes the maintenance of acidic pH and the exportation of metabolites generated in the lysosome to facilitate their utilization by other organelles or compartments in various aspects of cellular metabolism[22,23]. These membrane proteins (for example, LAMP1) are heavily glycosylated, forming a protective glycocalyx on the internal face of the limiting membrane. Intriguingly, sialic acid residues on LAMP1 play a role in the process of exocytosis, suggesting that the glycocalyx does more than simply provide a carbohydrate barrier to protect the limiting membrane from auto-catabolism[24]. However, the functions of the majority of lysosomal membrane proteins remain unknown at the present time[22,23]. Lysosomes can fuse with late endosomes, autophagosomes and phagosomes and thus are important for both maintaining cellular homeostasis and combatting infection[25]. They also form contact sites with other organelles (for example, mitochondria and the endoplasmic reticulum (ER)) where exchange of ions, lipids and other molecules takes place[26,27]. This is an area that requires more research, as it will no doubt yield major insights into lysosomal crosstalk with other organelles and provide a better understanding of how metabolites move out of the lysosome to be utilized in other cellular compartments[27]. Over the past 20 years, many additional functions of the lysosome have been identified, including nutrient sensing, lysosomal cell death pathways, plasma membrane repair and calcium signalling[28–31]. The lysosome therefore has emerged from the shadows of mundanity, having been previously viewed strictly as a 'housekeeping' organelle, out into the spotlight as a key cellular sensing and signalling hub[32]. There is no doubt that there is still much to learn about this enigmatic organelle, and it is interesting to note that many of the insights into lysosomal function have arisen, and continue to arise, from studying a family of rare inborn errors of metabolism, the LSDs[6].

Box 1 | **The legacy of orphan drug legislation**

In the 1970s, it became apparent that the drug discovery and development process favoured therapies for common diseases, leaving rare diseases without treatment. In 1979, a cross-sector report was published in the USA entitled 'Significant Drugs of Limited Commercial Value', which paved the way for the 1983 Orphan Drug Act[188]. The problem identified in this report was that the potential financial return for the pharmaceutical industry for rare disease products was perceived to be too small to be commercially viable and so acted as a considerable disincentive[194,195]. The Orphan Drug Act therefore aimed to solve this problem by providing a number of primarily financial incentives (including tax credits, periods of market exclusivity and protocol assistance) that would encourage the pharmaceutical industry to develop innovative new products for rare diseases[196].

Another key factor was the creation of the National Organization for Rare Disorders (NORD), which works on behalf of and in partnership with patients with rare diseases. This group was able to lobby very effectively, along with other interest groups, to counteract President Ronald Reagan's intention to veto the orphan drug legislation[194]. Their success led to the Orphan Drug Act being signed into law in 1983. It was amended in 1984 to define the prevalence of rare diseases. The first approved drug to benefit from this legislation was for porphyria (haematin), and a trickle of other approved products followed. However, things radically changed when several companies used the orphan products legislation as the core of their business model, including Genzyme and BioMarin, which developed some of the blockbuster biologic therapies for lysosomal diseases (discussed in detail elsewhere in this Review)[9]. The high prices and profitability of orphan products was not envisaged when the legislation was crafted and has been an unintended consequence. However, it could be argued that without this financial incentive, there would be far fewer products approved and far less pharmaceutical industry activity in the orphan disease space.

After the Orphan Drug Act was passed in the USA, it was several years before similar legislation was passed by the European Union (1999), creating an orphan drug programme (Regulation EC 141/2000). Currently, 28 member states come under this legislation, encompassing more than 500 million people. Japan passed similar legislation in 1993, as did Australia in 1998, and several other countries have also followed suit[194]. Orphan drug legislation has been a major success, catalysing innovative new therapies for many rare diseases, substantially benefiting patients and their families. In addition, it has led to the development of a new generation of pharmaceutical companies that develop rare disease therapeutics catalysed by the legal framework first created in 1983 and the financial incentives central to this legislation.

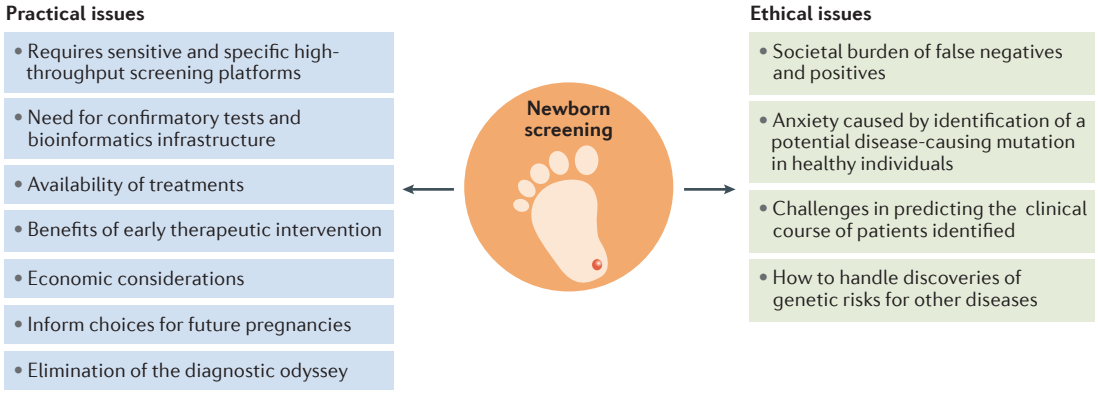

Figure 1 | **The complexity of practical and ethical issues raised by newborn screening.** Practical considerations are shown in blue, and ethical issues are shown in green. The use of dried blood spots from the newborn baby is the paradigm ideally suited to such screens. Some of the factors influencing the successful introduction of a newborn screen for a given inherited disease are illustrated to show the complexity of such screens and the number of factors that influence the adoption of a screen into routine practice.

Lysosomal storage disorders

LSDs are a group of over 70 inherited metabolic disorders caused by mutations in genes encoding proteins involved in different aspects of lysosomal homeostasis[1]. Most are inherited as autosomal recessive traits, although a small number are X-linked (for example, Fabry disease and mucopolysaccharidosis II (MPSII; also known as Hunter syndrome))[1]. Although individually rare (orphan or ultra-orphan), they collectively affect 1 in 5,000 live births and most commonly present as paediatric neurodegenerative diseases[1]. Peripheral tissues and/or organs can also be affected; thus, these diseases can frequently be multi-system disorders. In isolated human populations and those with high consanguinity rates, the frequency of these diseases can be much higher. Some at-risk populations have introduced a number of successful preventive programmes[33,34] that are discussed in more detail below.

The majority of LSDs are the result of defects in lysosomal enzymes[35], lysosomal membrane proteins[36] and proteins involved in the wider transport machinery that delivers enzymes to the lysosome[37], proteins that help lysosomal hydrolases interact with lipid substrates (activator proteins)[38] or proteins that export cargo from the lysosome[1] (TABLE 1). LSDs are characterized by the accumulation (so-called 'storage') of non-degraded substrates in the lysosome, with each disease having its own biochemical fingerprint of stored metabolites[39]. Many of these diseases were clinically described over a century ago and began to be classified on the basis of the biochemical nature of the storage material in the 20th century. The genes responsible for some of these diseases were more recently identified. For example, the sphingolipidoses encompass diseases in which sphingolipids are stored, typically as a result of mutations in genes that encode the enzymes involved in sphingolipid catabolism[38]. The situation is actually much more complex than this, as secondary storage metabolites frequently build up[40]. Another way of grouping these diseases is based on the underlying mechanism leading to storage (for example, enzyme deficiency, transport defect, etc.)[41] (TABLE 1).

LSDs provide a unique window into fundamental cell biology. By studying what happens when the gene is faulty, we can better understand how the gene regulates key aspects of lysosomal homeostasis in healthy cells. However, we still do not fully understand how a specific mutation in a patient leads to their individual rate of disease progression and precise clinical manifestations. Most patients are compound heterozygotes (that is, they inherit a different mutation in the same gene from each parent), and it is not uncommon for siblings (including twins) that harbour identical mutations to display discordance[42,43]. A greater understanding of modifier genes, epigenetic modifiers, infectious diseases and environmental and/or dietary factors that affect clinical presentation will no doubt emerge in the coming decade and may well offer novel routes for treating these diseases. Another important feature of LSDs is that, through convergent pathogenic mechanisms, they can aid our understanding of pathogenesis in more common neurodegenerative diseases, infectious diseases, cancers and other inherited diseases[44–48]. Therapies developed for LSDs may thus have unanticipated utility beyond the LSD field[48–50]. Most notably, being a carrier for a mutation causing Gaucher disease represents the highest genetic risk factor for developing Parkinson disease[50–54].

LSDs have a major advantage over more common neurodegenerative disease fields in that there are numerous large and small animal models in which pathogenesis and experimental therapies can be studied. These models have greatly facilitated the successful translation of therapies into the clinic[55,56]. This is probably one of the most important factors that underpin the remarkable translational success and burgeoning translational activity in this family of orphan diseases.

One truly remarkable aspect of LSDs is that virtually every cell in the body has a lysosomal system, and that system is defective in any given LSD. However, not every cell type and system in the body may be affected and certainly not to the same degree. This divergence can be due to a number of factors, including the differential

Table 1 | **Summary of the molecular mechanisms leading to lysosomal storage disorders**

Molecular defect	Examples of diseases	Defective protein	Refs
Lysosomal hydrolase	• Gaucher • Tay–Sachs • Wolman • Pompe	• GBA • β-Hexosaminidase A • Acid lipase • GGA	35
Lysosomal hydrolase trafficking	Mucolipidosis type II	*N*-acetyl glucosamine phosphoryl transferase	35,217,218
Lysosomal hydrolase post-translational modification	Multiple sulfatase deficiency	All known sulfatases	219
Lysosomal hydrolase protection	Galactosialidosis	β-galactosidase and neuraminidase	220
Lysosomal membrane protein	• Niemann–Pick type C1 • Danon • Mucolipidosis type IV	• NPC1 • LAMP2 • MCOLN1	28,221–224
Non-enzymatic soluble lysosomal protein	• Niemann–Pick type C2 • Neuronal ceroid lipofuscinosis	• NPC2 • CLN5	225–227
Miscellaneous	Neuronal ceroid lipofuscinosis	CLN4 and CLN7	228–230

CLN, ceroid-lipofuscinosis, neuronal; GBA, β-glucocerebrosidase; GGA, α-glucosidase; LAMP2, lysosome-associated membrane glycoprotein 2; MCOLN1, mucolipin 1 (also known as TRPML1); NPC1, Niemann–Pick C1 protein.

biochemistry of distinct cell types, differential turnover rates of substrates, catabolic enzyme redundancy, adaptive changes to counteract the primary defect, whether cells are regenerative or terminally differentiated and the stage of fetal development when storage reaches a threshold to trigger cell dysfunction. In order to treat an LSD effectively, this knowledge is vital, as the therapies in question need to be able to access the key anatomical sites and cell types affected in any given disorder.

Clinical manifestations of LSDs

Lysosomal diseases exhibit a range of clinical manifestations and have recently been reviewed elsewhere[6]. However, a few general points that are particularly relevant to therapeutic development are discussed here. Most affected individuals appear healthy at birth, and dysmorphia is generally confined to LSDs that affect the extracellular matrix and bone, such as the mucopolysaccharidoses[57]. Suspicion of an LSD is usually triggered by evidence of visceral disease (for example, hepatosplenomegaly in Gaucher and Niemann–Pick type B diseases or acute postnatal liver disease in Niemann–Pick type C disease) or failure to achieve developmental milestones due to the effects of storage in the CNS (for example, in Tay–Sachs disease)[58]. Behavioural changes can also raise suspicion of an LSD and can include psychiatric symptoms, particularly in later-onset forms of these diseases[59].

Any individual LSD manifests with a set of symptoms, which in combination define that particular disease but are typically not unique to the LSD in question. For example, seizures are common clinical signs in several LSDs affecting the brain; the aetiology may be different in terms of the pathogenic mechanism causing the seizures, but the seizures themselves are not usually restricted in their clinical presentation to LSDs. Indeed, at this level, raising seizure thresholds using conventional drugs can be an effective treatment. Use of the current pharmacopoeia is therefore the bed-rock of current clinical management (palliative pharmacotherapy) for most patients with LSDs and should not be overlooked when thinking about developing more specific disease modifiers, as management of symptoms contributes enormously to the quality of life of patients and their families[60].

The diagnostic odyssey

For most patients with LSDs, it takes several years to achieve a diagnosis. This diagnostic odyssey may seem somewhat esoteric to the reader of an article focusing on LSD therapies. However, if a company develops a game-changing therapy, yet the patients remain undiagnosed, drug discovery efforts will be largely wasted. Indeed, a more detailed knowledge of how easily patients with a given LSD can be diagnosed, how well their natural history is understood and whether any outcome measures have been validated for clinical trials should be considered when deciding which disease to target (BOX 2).

Diagnosis, therapy, access to therapy and health-care economics are all confounders in the complex and challenging journey from idea to product. Key players in this process are the patient organizations, as they not only support newly diagnosed families but also promote commercial interest in 'their disease'. These organizations are also major drivers of natural history studies and support the creation and management of patient registries.

Diagnosis, prevention and screening

The diagnosis of an LSD is a devastating event for a family and begins with an often protracted diagnostic odyssey, followed by a journey that leads to morbidity, reduced quality of life, partial or total dependency and invariably premature death, often in childhood, adolescence or early adulthood. The current diagnostic tests are based on various approaches, including lysosomal enzyme level measurements, cellular assays and mutation analysis, with a greater emphasis being placed on molecular diagnostics[61]. A diagnostic delay of several

Box 2 | **Factors to consider in drug development for LSDs**

It is not uncommon for an academic group or commercial enterprise to be faced with the question of which lysosomal storage disorder (LSD) they should target for a new therapeutic development programme. Below are the key factors that need to be considered carefully and used to help steer the development programme in a direction that has a fighting chance of success.

- Is there a translationally relevant model system available?
- Does the model system respond to the therapeutic intervention?
- Are there clinical natural history data or a registry available?
- Are there agreed severity scales for patient assessment?
- Are the patients fairly easy to diagnose?
- Are there enough patients diagnosed to consider a conventional clinical trial?
- Can trials be sufficiently powered to demonstrate efficacy?
- Are there any tools available to stratify patients for clinical trials?
- Are there agreed outcome measures likely to respond to a therapy?
- Can the outcome measures be measured quantitatively and easily in multiple centres?
- Are clinical changes in response to treatment likely to manifest within the 1–2 year time frame of a clinical trial?
- Are there validated clinical end points, biomarkers or surrogate end points?
- What is the definition of therapeutic success and what are the views of the patients and their families?
- Are the primary outcome measures relevant to patient quality of life?
- Is there a patient association to support patients and inform translational activity?
- Is the therapeutic product supply ensured and scalable?
- Is manufacturing in place?
- Has a compassionate use provision been considered and planned for?

years is unfortunately very common, and frequently other children are born to parents before the first presenting child (the index case) is diagnosed[62]. The reasons for diagnostic delay are multifactorial but often result from a lack of clinical awareness due to the rarity of LSDs[63]. The presenting clinical signs can involve multiple organ systems, so patients may be seen by several specialists who may fail to see the 'bigger picture' that the patient has a complex, multi-system, inherited, rare disease that requires urgent diagnosis. A successful diagnosis involves close links between multiple clinical and research specialists, including clinicians, scientists, bioinformaticians and genetic counsellors[64]. The pros and cons of several currently used diagnostic methods have recently been reviewed[12].

Several health-care systems worldwide have developed specialist referral centres where patients with LSDs can be diagnosed and optimally managed by expert physicians who see a large number of patients with LSDs in their clinical careers. They also serve as the major clinical centres for conducting clinical trials and often run multiple trials for companies working in the LSD space. In health-care systems where patients are seen and managed locally, diagnostic delay is typically longer, and clinical management unfortunately is not always optimal. The situation for patients with adult-onset disease is even less satisfactory, as most patients experience repeat misdiagnoses that can span several decades. This is because the presenting signs in adults frequently resemble more common neuromuscular, neurodegenerative or psychiatric diseases. In addition, LSDs are often erroneously viewed as exclusively paediatric disorders; as a consequence, many patients with adult-onset disease remain undiagnosed, and tests to investigate the possibility of an LSD are rarely commissioned outside of specialist referral centres. Raising awareness more generally in the training of medical students and health professionals (neurologists, ophthalmologists, hepatologists, haematologists, etc.) therefore needs to be a priority[63]. Importantly, rapid diagnosis would allow the parents of an affected child to make an informed decision about subsequent pregnancies. One practical aid to prevention would be to introduce newborn screening, with the objective of diagnosing the first affected child before any subsequent pregnancies[62] (BOX 3). Although this may appear straightforward, it is actually surprisingly complex to achieve in practice and raises a number of ethical issues[11,65] (FIG. 1).

Approaches to therapy

In the current setting of considerable diagnostic delay and a lack of newborn screening and/or prevention strategies in place, the need for therapeutic intervention remains high. The monogenic nature of LSDs and the detailed knowledge of the function of many of the proteins defective in these disorders (TABLE 1) provide multiple therapeutic intervention points. As with all diseases, the primary pathological trigger (in this case, the inherited mutation) initiates a pathogenic cascade that is often remarkably complex (FIG. 2). It is reasonable, therefore, to anticipate that therapies targeting the apex of this cascade will be the most clinically effective. The various therapeutic approaches are summarized in TABLE 2. Therapies can target distinct biological processes in different cellular organelles, beyond the lysosome (FIG. 3), and thus the therapeutic agent in question must access the appropriate cellular compartment or be engineered to target it correctly.

Therapies for LSDs fall into two categories, the first being disease-specific therapies, and the second being therapies that target convergent elements of the pathogenic cascade (downstream targets) and thus may be applicable to more than a single disorder. Disease-specific therapies have the disadvantage that, by definition, they can only be used in a small subset of patients with LSDs. However, their major advantage is that they have the potential to be the most effective. By contrast, therapies targeting downstream processes have the advantage of being potentially applicable to multiple LSDs but are at a disadvantage because they are more likely to be disease modifiers or adjunctive therapies, and within the pathogenic cascade, they are several steps removed from the primary defect (although there are exceptions discussed below, that is, compounds affecting proteostasis, known as proteostasis modifiers). Below, therapeutic approaches already in clinical practice or currently being explored for LSDs are reviewed.

History of LSD therapies

It was appreciated early on by Hers and de Duve[66,67] that most LSDs result from a lysosomal enzyme deficiency, and this observation provides the rationale that

Proteostasis
The integrated process of protein synthesis, folding, trafficking and catabolism. Modulating this process is a therapeutic strategy for treating protein-misfolding diseases, including lysosomal storage disorders.

Proteostasis modifiers
Drugs that increase the activity of the protein-folding machinery of the cell to aid folding of misfolded mutant proteins.

underpins the majority of currently approved therapies[35,68]. The cell biology of lysosomal enzymes is complex but highly favourable from a therapeutic point of view. Through the pioneering work of Neufeld[69,70], it was established that lysosomal enzymes mediate a process called cross-correction. Lysosomal enzymes, like other cellular glycoproteins, are synthesized in the ER and then move through the Golgi, where their *N*-glycans are processed, and they are often further modified to carry a mannose-6-phosphate (M6P) residue that targets them to the lysosomal system[68,71]. However, a proportion of the enzyme is released from the cell as a soluble glycoprotein that can be taken up by neighbouring cells by binding to surface receptors (for example, the M6P receptor) and subsequently enters the endocytic system and is delivered to the lysosome where it can function. Pathways independent of M6P also occur[72]; for example, LIMP2 is the protein that escorts the lysosomal enzyme β-glucocerebrosidase (GBA) to the lysosome and, when deficient, causes Gaucher disease[73].

The earliest attempt to treat an LSD caused by an enzyme deficiency used intravenous injection of hexosaminidase into a patient with Tay–Sachs disease, which did not cross the blood–brain barrier and thus was not effective[74]. This was followed by transplantation approaches using donor haematopoietic stem cells administered through the invasive process of bone marrow transplantation (BMT), also termed haematopoietic stem cell transplantation (HSCT). Indeed, as a therapeutic modality it is one of the earliest biologic therapies to be put into routine clinical practice[75], with the first report in a patient with MPSI (also known as Hurler syndrome) in 1981 (REFS 76,77). However, HSCT has a number of important limitations. It historically required identification of a suitably matched donor and immunosuppression of the recipient to prevent graft rejection; in addition, the procedure is associated with high morbidity and mortality, with very mixed clinical outcomes in LSDs[78]. If the transplant is conducted before 1 year of age, clinical outcomes are better[79,80], which

Box 3 | **Screening and prevention of LSDs**

Screening has two potential purposes. The first is to identify affected individuals as early as possible to instigate effective therapy, and the second is to provide reproductive choices to the parents to take preventive action to avoid the birth of other affected individuals. Screening and prevention are therefore intricately linked.

Newborn screening was pioneered by Robert Guthrie (1916–1995) to identify the inborn error of metabolism phenylketonuria and instigate rapid treatment in affected individuals[197]. His innovation was to establish a routine procedure to take a drop of blood from all newborn babies, dry it onto filter paper (known as Guthrie cards) and test the blood in the laboratory. Fortunately for the lysosomal storage disorder (LSD) community, dried blood spots retain active lysosomal enzymes and intact storage metabolites and thus are very amenable to prospective and retrospective analysis[198–203]. This means newborn screening based on dry blood spot analysis would, in principle, be practical for LSDs. In addition, molecular–genetic analysis can be conducted on patient blood samples.

Devising a biochemical or genetic screen is technically fairly straightforward[11]. However, genetic screens cannot easily distinguish a benign polymorphism in a potentially disease-causing gene from an actual disease-causing mutation, so we run the risk of branding the newborn baby as an individual with a LSD with no certainty that they will ever become symptomatic in their lifetime, potentially restricting their access to health insurance in those with private health-care provision[204]. The problem is further compounded by the fact that penetrance for many LSDs is not 100%, even for diseases with known disease-causing mutations[43]. Biochemical screens (biomarkers) that detect storage or downstream products of storage may be better predictors of actual disease burden than mutation analysis and are being explored in several LSDs[205–210]. Increased relative lysosomal volume of circulating cells can also be used; an expanded lysosomal compartment raises the suspicion that the patient may have an LSD and can trigger subsequent specific testing to provide a definitive diagnosis[206]. Screening in discrete populations with a small number of highly penetrant mutations present at high frequency has currently proved to be the most effective. These are typically either geographically isolated populations that have a founder mutation or ethnic groups with high carrier rates for mutations in one or more LSD-causing genes. Two examples of high prevalence in geographically isolated communities are Niemann–Pick type C disease in Nova Scotia in Canada[211,212] and type 3 Gaucher disease in Norrbotten in the north of Sweden[213].

The Ashkenazi Jewish community is an ethnic group that has high carrier rates for several LSDs, and they have introduced preventive programmes that illustrate how successful this approach can be[34,214]. Tay–Sachs disease is caused by mutations in the lysosomal enzyme β-hexosaminidase A (α-subunit deficiency), leading to GM2 ganglioside storage[58,215] (TABLE 1). The disease has a very high carrier frequency, involving a limited number of common mutations within this community[216]. Screening, based on a combination of residual enzyme activity measurements and mutation analysis, is therefore practical and has proved to be highly effective, reducing cases by 90%[214]. The patient advocacy and support organization National Tay–Sachs and Allied Diseases (NTSAD) is celebrating its 60th anniversary this year and was established to facilitate carrier testing for the Jewish community, and although its remit has broadened since then, it continues to support a quality control programme that validates the enzyme test results provided by the various screening laboratories that offer this service. Paradoxically, this success has led to the erroneous belief that Tay–Sachs disease has been eradicated, which is not the case, and unfortunately affected children continue to be born to parents in the general population who are unaware that they are carriers of Tay–Sachs mutations.

With pre-implantation genetic diagnosis (embryo selection followed by *in vitro* fertilization) now a routine procedure in many countries, subsequent pregnancies could be guaranteed to be free from these devastating genetic diseases, but this can only happen if the family realizes it is at risk in the first place. It is easy to get carried away with the elegance of the underlying science and promise of innovative new therapeutic approaches in LSDs, and we tend to overlook the simple truth that 'prevention is better than cure'.

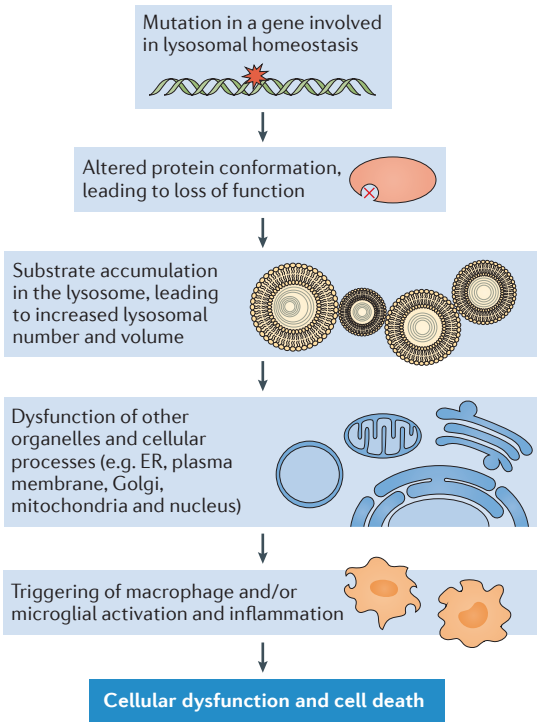

Figure 2 | **The pathogenic cascade in lysosomal storage disorders (LSDs).** Each major step in the pathogenic cascade is illustrated, starting from the genetic defect. The mutant protein, frequently a lysosomal enzyme, is either not transcribed or is transcribed but is subject to clearance via the quality control process. Any enzyme remaining has compromised function, leading to reduced residual enzyme activity. The consequence of protein dysfunction is the build-up of substrates in the lysosome, leading to expansion of the acidic compartment. Through ill-defined mechanisms, other secondary effects of storage are manifested in other cellular organelles, leading to 'collateral damage' in various aspects of cellular homeostasis. Finally, the innate immune system is triggered, leading to inflammation both in peripheral tissues and in the brain in neurodegenerative LSDs. ER, endoplasmic reticulum.

again reinforces the need for early diagnosis. Indeed, this is a major justification for newborn screening for HSCT-responsive LSDs (FIG. 1). However, despite these limitations, it is an effective disease modifier for some LSDs[78,79]. As allogeneic BMT is a medical procedure, it is somewhat anomalous — it has never undergone the rigours of regulatory approval and thus falls outside the classical therapeutic development framework. In the modern era the focus has shifted to using autologous HSCT to isolate progenitor cells from the patient themselves that can be transduced *ex vivo* with a wild-type copy of the defective gene (gene therapy), to convert the haematopoietic system into an enzyme producing and/or secreting 'factory' (see below). Other cell-based therapies, such as neural stem cell therapies, have shown some efficacy in animal models and have been evaluated in clinical trials in a very small number of LSDs[81–83], but are not yet approved for any LSD.

Disease-specific therapies for LSDs: biologics

When we consider the use of biologic therapies in the modern era, the 'blockbuster' therapeutic monoclonal antibodies immediately come to mind. However, most currently approved LSD therapies are also biologics. As LSDs are monogenic diseases, the two most cogent therapeutic approaches are to mitigate the effects of the faulty gene by introducing a fully functional gene[84–89] (TABLE 2), or to replace the defective protein by administering a recombinant wild-type protein into the patient's circulation or delivering it directly to the CNS via a device[90–94].

Before discussing these approaches in more detail, it is important to remember that most LSDs involve storage and pathology in the brain, as well as in peripheral tissues and/or organs, and thus the greatest technical challenge is how to deliver therapies to effectively treat all organs and tissues using a single therapeutic strategy. This issue remains largely unresolved, which means that for the current generation of patients, either the CNS remains untreated or highly invasive methods have to be employed to deliver protein therapies to the brain. There can be little doubt that targeting the brain and leaving the periphery untreated or vice versa will be unsatisfactory in the long term. As a consequence, although we can change the natural history of these diseases by correcting or partially correcting one set of clinical phenotypes, the extended lifespan of the patient allows for the emergence of new symptoms.

Targeting the gene. The objective of this approach is to introduce, either by direct injection into the circulation or the brain, a wild-type version of the faulty gene into the affected individual, currently through the use of adeno-associated virus (AAV) or retroviral or lentiviral vectors[89] (TABLE 3). There are still concerns about how to ensure therapeutic levels of gene expression without leading to excessive production of the gene product in question, as overexpression of lysosomal genes could have deleterious consequences for cell function.

The discovery that AAV9 can be administered intravenously and can correct defects in the periphery and cross the blood–brain barrier raises the prospect of much less invasive gene therapy delivery in the future[95]. An alternative strategy that requires lower amounts of vector is to perform HSCT *ex vivo* gene therapy by introducing autologous corrected HSCs back into the patient's circulation[89]. Macrophage lineage cells can then migrate from the bone marrow to the CNS, differentiate into microglia and serve as a source of a fully functional enzyme. Indeed, this is the basis for the beneficial effects of HSCT without gene correction in some CNS disorders. However, the numbers of cells that migrate into the brain are fairly small; thus, this is not a very efficient process and is of limited clinical efficacy[96].

In regard to gene therapy, LSDs are viewed as 'low-hanging fruit'. This is because very small increases in the residual function of a mutant protein can make a major difference to the natural history of these diseases. If, for example, we consider Tay–Sachs disease (TABLE 1), we know that the lower the residual enzyme activity

Table 2 | **Summary of the therapeutic approaches to treating lysosomal storage disorders**

Therapeutic approach	Step in pathogenic cascade being targeted or replaced	Stage of development	Examples of diseases treated	Therapeutic agent or commercial product	Refs or clinical trial
Gene therapy for soluble proteins	Defective gene	Animal model POC	Tay–Sachs	β-Hexosaminidase A and B	231
Gene therapy for membrane proteins	Defective gene	Animal model POC	Niemann–Pick type C	NPC1 (adeno-associated virus)	99
Gene editing	Defective gene	NA*	NA	NA	102
Protein replacement	Defective protein	Multiple enzyme replacement therapies approved for multiple diseases	• Gaucher • Fabry • Pompe • MPSI • MPSII • MPSIV	Multiple approved ERTs	117
Proteostasis modifiers	Defective protein	Clinical trials in Niemann–Pick type C disease	HSP70 inducer drug	Arimoclomol	161
Small-molecule enzyme enhancers or chaperones	Defective protein	Approved	Fabry	Migalastat	131
		Clinical evaluation	• Sandhoff • Tay–Sachs	Pyrimethamine	232
			Gaucher	Ambroxol	125
Substrate reduction	Substrate accumulation	Approved	Gaucher	Miglustat and eliglustat	151,233
			Niemann–Pick type C	Miglustat	152,234
		Clinical exploratory studies/trials	Fabry	Lucerastat	154
			• Fabry • Gaucher • Parkinson	Ibiglustat (Genz-682452)	235,236 NCT02228460, NCT02843035 and NCT02906020
			MPSIIIB	Genistein	156
Ca^{2+} homeostasis	Secondary defect in Ca^{2+} homeostasis	Animal model POC	Niemann–Pick type C	Curcumin	237

HSP70, heat shock protein 70; MPS, mucopolysaccharidosis; NA, not applicable; POC, proof of concept. *Gene editing not yet at the stage of *in vivo* proof of concept.

(β-hexosaminidase) a patient has as a result of a mutation, the more rapidly the storage substrate builds up (in this case, GM2 ganglioside), leading to an aggressive form of the disease with death in infancy or early childhood. On the other hand, patients with a juvenile-onset form of the disease present at a later age and live longer, while patients with adult-onset disease may not develop clinical signs until well into adulthood and have a fairly normal life expectancy, albeit with the burden of a disabling neuromuscular disease. Remarkably, the differential levels of residual enzyme between different ages of clinical onset are actually quite subtle[97]. Therefore, even fairly inefficient gene therapy could generate sufficient wild-type, fully functional enzyme to convert a severe disease into a milder disease, assuming diagnosis is rapid and that the therapy can be introduced pre-symptomatically or at least very early in the clinical course of the disease, before many irreversible neuropathology events have occurred.

There are currently multiple preclinical studies that show a benefit of gene therapy in both small and large animal models, and some of these have moved in to phase I or phase I/II safety studies in patients with LSDs (TABLE 3). For example, two forms of neuronal ceroid lipofuscinosis (NCL) (CLN2 and 6) are in phase I and I/II trials, using AAV-based vectors delivered via intracranial or intrathecal routes. There are also multiple phase I and phase II trials in MPS diseases (MPSII, MPSIIIa and MPSIIIb) using either retroviral *ex vivo* gene correction of HSCs for MPSII or AAV vectors delivered intravenously or intracranially for MPSIII (TABLE 3). The fact that some gene therapy vectors are already approved[98] for clinical use for other indications will no doubt accelerate the development and regulatory process for gene therapy for LSDs.

A long-standing and prevailing view in the LSD field has been that gene therapy will only work for soluble enzymes because of cross-correction. The reason for this view is that if transduction in the human brain is inefficient, then secretion of soluble enzyme from transduced cells will serve as a source of enzyme that can be taken up by neighbouring non-transduced cells, which cannot happen in the case of membrane proteins. However, recent findings in preclinical studies have shown that the introduction of the lysosomal membrane protein

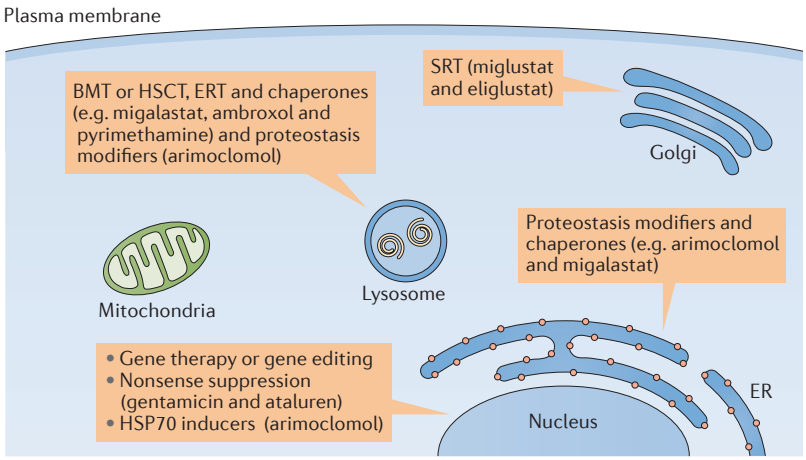

Figure 3 | **The major sites of action of the current lysosomal storage disorder therapeutics.** Gene therapy and gene-editing approaches aim to introduce a functional gene or to correct the defective gene. Enzyme replacement therapies (ERTs) deliver a functional enzyme to the lysosome, as does bone marrow transplantation (BMT) or haematopoietic stem cell transplantation (HSCT). Chaperones can stabilize the mutant enzyme and partially restore catalytic activity in the endoplasmic reticulum (ER) and in the lysosome. Substrate reduction therapy (SRT) drugs inhibit glucosylceramide synthase on the outer leaflet of an early Golgi compartment. Finally, the proteostasis modifiers that induce heat shock protein 70 (HSP70) stabilize transcription factors in the nucleus, improve lysosomal enzyme expression and translation in the ER, reduce lysosomal membrane permeability and improve lysosomal function.

NPC1 (a large multi-pass transmembrane protein that is deficient in most patients with Niemann–Pick type C disease) into *Npc1*-null mice using AAV vectors led to a clinically relevant benefit, suggesting that gene therapy for lysosomal membrane proteins will ultimately be a viable approach[99]. This is currently a very active preclinical area of research and offers some hope to patients suffering from membrane protein deficiencies, which account for a large number of monogenic human diseases. Other approaches to tackle genetic defects directly include stop-codon-readthrough technologies (nonsense suppression) (FIG. 3) that use small molecules to overcome mutations that would result in in-frame premature termination codons. Classically, drugs such as the aminoglycoside gentamycin were used for proof of concept[100], with many more compounds now under evaluation or development, which have been recently reviewed comprehensively in the context of LSDs[101]. To date, 17 proof-of-concept studies have been conducted *in vitro* and in some murine models across multiple LSDs, primarily using gentamycin. Screens to identify proprietary molecules have yielded, for example, ataluren (Translarna; PTC Therapeutics), which has shown efficacy *in vitro* and in a mouse model of CLN1 and was given conditional approval by the European Medicines Agency (EMA) for the treatment of Duchenne muscular dystrophy[101]. Genome-editing techniques are improving in their reliability *in vivo* and will no doubt be moving towards the clinic for LSDs in the future, with the prospect of removing or correcting deleterious mutations in tissues of the body (TABLE 2). Safety concerns are the biggest hurdle to overcome, along with addressingtarget organs effectively[102,103]. This rapidly evolving field has been reviewed recently[104].

ERT. The first ERT (GBA) was pioneered for type 1 Gaucher disease by Brady and colleagues[10,105] at the US National Institutes of Health. The first product was placentally derived (Ceredase; Genzyme) and was approved by the US Food and Drug Administration (FDA) in 1991 following a small, open-label clinical trial. The trial design was very simple with straightforward clinical outcome measures. The end points included reduced liver and spleen volumes and improved haematological parameters (for example, improvement or correction of anaemia and thrombocytopaenia). We have yet to see another ERT with such efficacy; hence, trials tend to involve greater numbers of enrolled patients and be placebo controlled, and multiple trials are often required. A good example of a modern ERT trial is acid sphingomyelinase ERT for Niemann–Pick type B disease including a phase III trial[106,107]. It could be argued that the current regulatory clinical trial requirements are too strict for rare diseases, and interestingly, recent guidelines for industry include less stringent phase III requirements (see Further Information). The current situation in part reflects the history of the drug approval process, which has not previously differentiated between the divergent needs of rare and common diseases — there is a single process to deal with these two very different disease sectors. However, as the orphan disease therapeutic space expands, the system will likely evolve as more experience is gained, leading to changes in the regulatory framework over the next decade.

Returning to ERT for Gaucher disease, the placental enzyme was replaced with a recombinant enzyme expressed in Chinese Hamster Ovary (CHO) cells (Cerezyme). This remarkable translational achievement and its catalysis of the development of other therapies for LSDs have been extensively reviewed following Brady's death in 2016 (REFS 108–110). The clinical efficacy and commercial success of ERT for Gaucher disease catalysed the development of ERT products for other LSDs. Furthermore, companies have developed multiple ERTs for Gaucher disease alone, with three products on the market, along with products marketed as biosimilars[111]. Several new ERTs for other LSDs are currently in clinical trials, including some that are administered directly to the CNS, such as tripeptidyl peptidase for the treatment of late infantile NCL (intracerebroventricular delivery) and intraventricular delivery of β-glucuronidase for MPSVII (TABLE 4). It is generally agreed that ERT products achieve varying degrees of benefit to patients depending on the stage in the disease course when treatment is initiated. Early intervention is key, as the disease can then be positively modified before the development of irreversible pathology. Another factor that explains differential responses to ERT is the development of antibodies to the infused therapeutic protein, which limits efficacy and reduces cost-effectiveness. A good example of this is in the neuromuscular LSD Pompe disease (also known as glycogen storage disease type II) (TABLE 1), in which

Table 3 | **Summary of clinical trials of gene therapy in lysosomal storage disorders that are in progress or completed**

Disease	Gene	Enzyme	Membrane protein	Vector	Delivery	*Ex vivo* transduction	Phase	Clinical trial
Type 1 Gaucher	*GBA*	+	–	Retrovirus	PBSCT	+	I	NCT00004294
Fabry	*GLA*	+	–	Retrovirus	HSCT	+	I	NCT00001234
Metachromatic leukodystrophy	*ARSA*	+	–	Lentivirus	HSCT	+	I/II	NCT01560182
				AAVrh10	i.c.	+	I/II	NCT01801709
Neuronal ceroid lipofuscinosis	*CLN2*	+	–	AAV2	i.c.	–	I	NCT00151216
				AAVrh10	i.c.	–	I/II	• NCT01414985 • NCT01161576
	CLN6	–	+(ER)	AAV9	i.t.	–	I/II	NCT02725580
MPSII	*IDS*	+	–	Retrovirus	Lymphocytes	+	I/II	NCT00004454
MPSIIIa	*SGSH*	+	–	AAV9	i.v.	–	I/II	NCT02716246
	SGSH and *SUMF1*	+	–	AAVrh10	i.c.	–	I/II	NCT01474343
MPSIIIb	*NAGLU*	+	–	AAV5	i.c.	–	I/II	ISRCTN19853672
Pompe	*GAA*	+	–	AAV9	i.m.	–	I	NCT02240407
				AAV1	i.m.	–	I/II	NCT00976352

Data are from ClinicalTrials.gov. AAV, adeno-associated virus; ER, endoplasmic reticulum; HSCT, haematopoietic stem cell transplantation; i.c., intracranial; i.m., intramuscular; i.t., intrathecal; i.v., intravenous; MPS, mucopolysaccharidosis; PBSCT, peripheral blood stem cell transplantation.

antibodies against lysosomal α-glucosidase (GAA) are generated[112,113]. Pompe disease is caused by a deficiency in GAA, leading to glycogen storage in multiple tissues and resulting in muscle weakness and wasting[35]. This disease, like many LSDs, is a clinical continuum, with patients with the severest form presenting soon after birth with muscle weakness and cardiac hypertrophy (the so-called infantile or 'classical' form of the disease). Later-onset forms also occur. ERT is an approved treatment for Pompe disease and involves intravenous infusions of recombinant GAA (Lumizyme (USA) or Myozyme (outside the USA); Genzyme). Recent reports of successful tolerization in patients with established immune responses to GAA suggest that this problem can be overcome, at least in some individuals[114]. However, lack of tolerance remains a concern with all ERT products and is an inevitable limitation of biologic therapies, particularly when infused into patients who are null for the gene product in question and thus lack tolerance to the protein[115]. One important consequence of the commercial activity in LSDs is that it has driven improvements in rates of diagnosis and the identification of patients as early as possible to rapidly initiate treatment[62]. The limitations of ERT include the high cost, invasive routes of delivery (most typically intravenous), infusion reactions owing to hypersensitivity in some patients and a lack of penetrance of the enzyme to key pathological sites (for example, the brain and bone)[9]. Nonetheless, there is no doubt that, as a class of biologic therapies for LSDs, ERT has substantially improved quality of life for many patients suffering from several LSDs and will continue to do so[105,116,117].

Disease-specific therapies for LSDs: small molecules

Because ERT has a number of limitations, other approaches have been sought to increase the activity of the mutant enzyme in LSDs using non-biologic therapies. The current approach is to use small-molecule drugs to augment enzyme activity, referred to as 'chaperone therapy'. Many disease-causing mutations in LSDs lead to the production of a protein that fails to pass the ER quality control machinery and thus never reaches the lysosome and is instead degraded via the proteasome. Other mutations lead to a protein that does reach the lysosome but is unstable and thus has a shorter half-life. The principle is to use a small-molecule active site inhibitor to stabilize the conformation of the mutant enzyme (potentially in the ER and in other cellular sites) to achieve a greater level of catalytic activity, thus increasing residual enzyme activity. By definition, small-molecule drugs will only work in patients with some residual enzyme function, and not all mutations are amenable to this approach. For each enzyme, a different chemical chaperone chemistry is needed, and thus these are disease-specific therapies. These small-molecule drugs are orally available and have the potential to be non-invasive, disease-modifying therapies that may also cross the blood–brain barrier.

Small-molecule chaperones. The use of active site inhibitors to augment enzyme activity is somewhat counterintuitive but is based on the finding that if a mutant, unstable enzyme binds a small molecule in its active site (for example, a substrate mimetic), the active site is stabilized and remains stable once the small molecule has dissociated[118,119]. This approach is therefore dependent on the fact that the off-rate for the inhibitor favours dissociation after the enzyme is stabilized, as otherwise the enzyme levels in the patient would be further reduced, not increased, due to sustained inhibition. Subinhibitory concentrations of these drugs are also used to favour enhanced enzyme activity. A major advantage of this approach is the wealth of

Table 4 | **Summary of ERT products approved (year of approval) or in clinical trials**

LSD	Enzyme deficiency	Receptor-mediated uptake	Route of administration	Year of first regulatory approval or clinical trial
Type 1 Gaucher	Glucocerebrosidase (three products)	Mannose receptor	i.v.	1991
MPSI	α-L-Iduronidase	M6P receptor	i.v.	2003
Fabry	α-Galactosidase (two products)	M6P receptor	i.v.	2003
MPSVI	*N*-Acetylgalactosamine-4-sulfatase	M6P receptor	i.v.	2005
MPSII	Iduronate sulfatase	M6P receptor	i.v.	2006
Pompe	α-Glucosidase	M6P receptor	i.v.	2006
MPSIVa	*N*-Acetylgalactosamine-6-sulfatase	M6P receptor	i.v.	2014
Wolman	Acid lipase	M6P receptor	i.v.	2015
Mannosidosis	α-Mannosidase	M6P receptor	i.v.	NCT01268358
MPSIIIa	Sulfamidase	M6P receptor	i.t.	NCT01155778
Late infantile neuronal ceroid lipofuscinosis	Tripeptidyl peptidase	M6P receptor	i.c.v.	NCT01907087
Metachromatic leukodystrophy	Aryl sulfatase A	M6P receptor	i.t.	NCT01510028
MPSIIIb	α-*N*-Acetylglucosaminidase	M6P receptor	i.c.v.	NCT02754076
MPSVII	β-Glucuronidase	M6P receptor	i.v.	NCT02432144
Niemann–Pick type A and B	Acid sphingomyelinase	M6P receptor	i.v.	NCT01722526

Data are from ClinicalTrials.gov. Combination therapy trials and enzyme replacement therapies (ERTs) engineered to cross the blood–brain barrier are in clinical evaluation in some LSDs. i.c.v., intracerebroventricular; i.t., intrathecal; i.v., intravenous; LSD, lysosomal storage disorder; M6P, mannose-6-phosphate; MPS, mucopolysaccharidosis.

small active site inhibitors known, many of which are imino sugar drugs[120]. Inhibiting hydrolases has been a very active area of research for many decades, and thus small-molecule chaperones are fairly straightforward to identify in conventional biochemical screens[120–128]. This approach works well in patient-derived cells that are exposed to molecular chaperones *in vitro*, but there are currently few animal models of LSDs that are engineered to express potentially 'chaperonable' mutant forms of the enzyme to fully test the efficacy of this approach *in vivo*. As a consequence, chemical chaperones have entered clinical trials without the demonstration of efficacy in an authentic preclinical animal model. The problem with the current generation of compounds is that it is quite challenging to devise a dosing regimen that favours improvement of enzyme function relative to inhibition. However, the recent approval of the active site inhibitor migalastat (Galafold; Amicus Therapeutics) in 2016 (REFS 129–131) by the EMA for the treatment of Fabry disease is a landmark for this approach[132]. This imino sugar drug involves a treatment regimen of every-other-day dosing in order to balance enzyme inhibition and/or stabilization to increase enzyme activity. Another chemical chaperone showing promise is the repurposed drug ambroxol, which is currently in investigator-led clinical studies for the treatment of neuronopathic type 3 Gaucher disease[125,133–137].

To overcome the limitation of active-site inhibitors, a new generation of chaperones that are allosteric enhancers is being developed[120,138,139]. Here, the small molecule binds away from the active site but induces a conformational change or stabilization that increases enzyme activity or extends half-life. A promising non-inhibitory compound to emerge from a chemical chaperone screen that has undergone medicinal chemistry optimization is NCGC607. This compound reduced lysosomal lipid storage and reduced α-synuclein levels in dopaminergic neurons derived from induced pluripotent stem cells from patients with Gaucher disease and Parkinsonism[140]. Although these drugs are not as far advanced through the development process as the active-site inhibitors, this approach holds the promise of conventional dosing regimens. However, both classes of small-molecule chaperones are disease-specific and mutation-specific and thus require careful testing of patient cells with a given mutation to assess their individual suitability for this approach[120]. This is an area of LSD drug discovery that therefore encompasses personalized medicine[141].

Non-disease-specific LSD therapies: small molecules

Substrate reduction therapies. The first small-molecule therapies to be approved for LSDs were substrate reduction therapy (SRT) drugs[142,143] (FIG. 3). SRT does not target the mutant enzyme but instead prevents the build-up of substrates[144,145]. An inhibitor of the biosynthesis of the substrate is used with the aim of balancing the rate of substrate biosynthesis to match the impaired rate of substrate catabolism. The greater the residual enzyme activity a patient retains, the more likely they are to benefit from this approach. The concept was first proposed by Radin[142,146] and was 'reduced to practice' in the glycosphingolipid (GSL) storage diseases. With the exception

Chemical chaperones
Drugs that either bind the active site of a mutant enzyme and stabilize it or are allosteric binders, binding away from the active site but still stabilizing the protein.

Substrate reduction therapy
(SRT). A small-molecule drug that inhibits the biosynthesis of substrates that are stored in a lysosomal storage disorder.

of galactosylceramide and its derivatives present in myelin, all other GSLs are synthesized through a common biosynthetic pathway that begins with the transfer of glucose to ceramide to form glucosylceramide (GlcCer)[147]. This reaction takes place on the outer face of an early Golgi compartment, and GlcCer is then the precursor for neutral GSLs and gangliosides. The formation of GlcCer is catalysed by glucosylceramide synthase (GCS), and this transferase is the target of two currently approved drugs, miglustat (Zavesca; Actelion Pharmaceuticals) and eliglustat (Cerdelga; Genzyme) (FIG. 3).

Miglustat is an imino sugar drug with glucose stereochemistry that acts as a short-chain ceramide mimetic by virtue of its alkyl chain and is a weaker GCS inhibitor than eliglustat, which is a longer-chain ceramide mimetic. Miglustat crosses the blood–brain barrier to some extent, whereas eliglustat does not[148]. Miglustat inhibits gastrointestinal tract disaccharidases, so its main side effect is osmotic diarrhoea[149]. Miglustat was first approved as a second-line treatment for type 1 Gaucher disease in 2002 by the EMA and in 2003 by the FDA and was approved by the EMA for the treatment of Niemann–Pick type C disease in 2009. Eliglustat was more recently approved as a first-line oral therapy for type 1 Gaucher disease by the FDA and EMA in 2014 (TABLE 2) and requires patient genotyping to ascertain their CYP2D6 status[148]. Other drugs that are metabolized by CYP2D6 may be contraindicated[150,151].

Miglustat was the first oral small-molecule therapeutic approved for an LSD, and both miglustat and eliglustat offer patients with type 1 Gaucher disease an oral-drug-based therapy as an alternative to intravenous ERT. A second imino sugar drug, lucerastat (Actelion; a miglustat analogue with galactose stereochemistry), with an improved side-effect profile has recently entered clinical trials for the treatment of Fabry disease[152–154]. Other CNS-penetrant SRT drugs are being developed with a view to treating CNS disease in GSL storage diseases. Currently, ibiglustat (Genz-682452; Genzyme) is in phase II trials for Fabry (NCT02226084), Gaucher (NCT02843035) and Parkinson diseases (NCT02906020).

SRT for other LSDs is very limited, although genistein is currently in clinical trials for MPSIIIB (also known as Sanfilippo syndrome, caused by an α-*N*-acetylglucosaminidase deficiency)[155]. Genistein is an isoflavone abundant in soya and functions as a broad-spectrum protein tyrosine kinase inhibitor that acts on epidermal growth factor (EGF) and insulin-like growth factor (IGF) receptors that regulate proteoglycan biosynthesis (proteoglycans are stored in MPS diseases). Genistein also modulates TFEB function, adding another dimension to the pharmacological properties of this phytoestrogen[156,157]. A phase III, randomized, placebo-controlled trial of high-dose genistein aglycone is fully recruited in Europe with children and adolescents less than 18 years of age with a proven diagnosis of MPSIII (EudraCT number: 2013-001479-18). The SRT approach is also being explored in animal models using antisense-oligonucleotide-mediated suppression of biosynthetic enzymes as an alternative to small-molecule inhibitors[158].

Proteostasis modifiers. Another strategy is to use small-molecule proteostasis modifiers, which increase the endogenous cellular response to stress and promote upregulation of the chaperone heat shock protein 70 (HSP70) to promote protein folding[159]. Unexpectedly, HSP70 was also found to interact directly with the anionic lipid bis(monoacylglycero)phosphate (BMP) found on internal vesicles within lysosomes, where it is key to creating a membrane environment compatible with sphingolipid catabolism[38]. HSP70 binds with high affinity to BMP and stabilizes acid sphingomyelinase, thereby enhancing its activity by prolonging its half-life. This function increases ceramide levels in lysosomal membranes and consequently reduces lysosomal membrane permeability[160]. The first cellular proof of concept for the use of HSP70 for treating an LSD was the discovery that cells from patients with Niemann–Pick type A or B disease could be corrected *in vitro*[160]. These studies were then extended to a panel of other LSD-derived cell lines, demonstrating broad efficacy[161]. In the same study, the first *in vivo* animal model data were presented, confirming phenotypic improvement in a mouse model of Niemann–Pick type C disease treated with the small-molecule drug arimoclomol[161], which induces HSP70 expression. Arimoclomol achieves HSP70 induction by stabilizing a transcription factor (activated heat shock factor protein 1 (HSF1)), which binds to heat shock elements in the promoters of heat shock-inducible genes, including *HSP70* (also known as *HSPA*)[162,163]. This drug therefore induces HSP70 only in cells that are already stressed and does not induce stress itself, as other HSP70 inducers have been recognized to do[164]. In addition to its potential use in LSDs, arimoclomol is also being investigated clinically for amyotrophic lateral sclerosis (ALS)[165] and sporadic inclusion body myositis[166]. A clinical trial of arimoclomol in Niemann–Pick type C disease is currently in progress (Orphazyme; NCT02612129). This drug has the potential to be used in multiple LSDs[161], as its mechanism of action is not disease specific. Another regulator of proteostasis, the drug celastrol, has also been evaluated in Gaucher disease and improved the effects of arimoclomol[167,168]. However, celastrol is a stress inducer and showed evidence of toxicity in some model systems in which it was tested[169].

Downstream modifiers: anti-inflammatories. Other non-disease-specific therapies include targeting inflammation. Innate immune activation of microglia along with recruitment of macrophages into the CNS is a common feature of many neurodegenerative diseases, including LSDs[170,171]. Some anti-inflammatory therapies or genetic manipulations have been trialled in animal models and suggest that inflammation not only is an active contributor to pathogenesis[170], but also represents a therapeutic target[172,173]. For example, synergy was demonstrated in a mouse model of Niemann–Pick type C disease when anti-inflammatory drugs were combined with miglustat (SRT) and the calcium modulator curcumin[173]. Clinical trials are needed to determine the extent of disease modification achievable, but this could be a promising area of research, particularly because

it involves drug repurposing using existing therapies marketed for treating chronic inflammatory diseases, thereby speeding the path to translation.

The involvement of the complement system, specifically C5a and its receptor C5aR, has recently been implicated in driving inflammation in genetic and pharmacologically induced models of Gaucher disease[174]. This leads to an autoantibody response that creates a vicious cycle of C5a generation and activation of C5aR, which in turn increases the synthesis of GlcCer, the main storage lipid in Gaucher disease. C5a was also found to be elevated in the sera of patients with Gaucher disease in the same study[174]. This raises the question of whether targeting C5aR may be a future strategy to treat Gaucher disease[174]. It will also be interesting to see if the complement pathway and autoimmune aspects are involved in the pathophysiology of other LSDs. It may be relevant that anti-ganglioside antibodies have been reported in a mouse model of Sandhoff disease[175], but the generality of this finding remains largely unexplored. Anti-GSL antibody pathophysiology is complex, as it is dependent upon the nature of the lipid environment in which the GSL epitope is present, an important finding arising from detailed studies of the autoimmune disease Guillain–Barre syndrome[176–178]. The presentation of GSLs by CD1d to invariant natural killer T cells (iNKT) is another immunological axis potentially involved in immune dysfunction in GSL LSDs and is an area of very active research in mouse models and patients[179–182]. The lysosome plays a key role in processing antigens that can be loaded onto CD1d and contains activator proteins that facilitate the loading of these lipids. As a consequence, lysosomal dysfunction can affect CD1d and iNKT cell biology, leading to changes in iNKT cell number and function[179]. However, there are important species differences, as CD1d localizes to the lysosome in murine models, whereas it localizes to late endosomes in humans. There are currently multiple models of how lysosomal dysfunction affects antigen presentation by CD1d and its impact on iNKT cell biology, but to date it remains unclear how changes in iNKT cell biology may contribute to LSD pathogenesis[179].

Therapeutic challenges and considerations

The discovery of enzyme cross-correction by Neufeld and the pioneering research by Brady led to Genzyme launching the first disease-specific LSD product on the market in 1992 (REF. 10). This collective academic and commercial achievement proved that a product for a small number of patients with a rare disease could be effective and profitable. The high level of clinical efficacy and remarkable improvement in outcomes for patients with Gaucher disease set a very high bar for everything that has followed, and few products, if any, have achieved the same degree of clinical success. This poses a number of problems in what is now a much more crowded commercial space where the 'low-hanging fruit' (that is, those LSDs without substantial CNS pathology) have largely been targeted, leaving the more complex diseases without effective therapies. The vast majority of these more complex diseases involve multiple chronic disease processes in multiple organ systems, which poses a challenge for not only therapy and/or therapeutic targeting but also diagnosis and effective clinical management.

Treating multimorbidity

One of the current areas of concern in health care is the increasing number of people in an ageing population living with multiple, typically chronic clinical conditions, a situation termed multimorbidity[183,184]. Currently, health-care systems tend to focus on single diseases affecting a major organ system, with medical training driving towards ever greater specialization. However, we are less well equipped to treat people living with multiple diseases. Multimorbidity is not unfamiliar to any expert clinician working in the LSD field. Indeed, it could be argued that LSDs and other inborn errors of metabolism are a microcosm of chronic multimorbidity. For example, the LSD Gaucher disease requires specialist knowledge in bone disease, haematological abnormalities (including myeloma) and neurological disease, which at the extreme end (type 2 disease) involves acute neurodegeneration[185–187]. When thinking about how to deal with multimorbidity in the general population, it may be timely to look at the provision of best practice in the rare disease field to help design appropriate health-care systems that can embrace multiple disciplines and deliver a more holistic approach to patient care. The specialist referral centres for LSDs in the UK and several other countries could be one model to emulate, as they are highly effective in diagnosing and managing these complex disorders.

Polypharmacology

The drugs developed to date (dominated by biologics, that is, ERT) have targeted the more prevalent LSDs and have generally avoided conditions with CNS disease, leaving a large unmet clinical need in the form of diseases involving the brain[188]. Strategies to deliver ERT to the brain from the circulation are being explored but have not yet delivered a therapeutic product that can cross the blood–brain barrier[189]. Furthermore, even effective ERTs do not access all peripheral tissues and organs equally, often resulting in differential efficacy in different aspects of pathology. For example, ERT for Gaucher disease does not fully manage bone disease[185]. Thus, the 'holy grail' will be the development of therapies that treat all compartments of the body effectively. We are certainly closer to achieving this in the modern era, but it unfortunately remains an unmet aspiration in terms of the currently approved therapies. Perhaps one of the major misconceptions in thinking about this goal, both academically and commercially, is that the 'holy grail' must be achieved with a single therapeutic agent. The practical reality of a holistic treatment for LSDs is much more likely to be delivered through the use of combination therapies (polypharmacology) tailored to each disease, with each therapeutic agent targeting unique aspects of the pathogenic cascade[144] (FIG. 2). It is worth reflecting on how successful and transformative for patients an analogous polypharmacological approach has been for managing HIV[190].

Clinical trial design: end points

Clinical trial design poses a number of general challenges, including clinical heterogeneity and a lack of tools for patient stratification. There is also the inevitability of conducting statistically underpowered trials and there is typically incomplete natural history data for many of these rare diseases. With each patient acting as his or her own control, the use of carefully curated, longitudinal natural history data collected before clinical trial enrolment would mitigate many of these confounders but is currently an aspiration and not a reality for most rare diseases. This is an area that patient advocacy groups are driving, with 'trial readiness' being the essential goal.

The selection of suitable end points is particularly challenging. In the past, biomarkers were used as primary end points in some clinical trials (for example, biochemical measurement of the stored GSL Gb3 in ERT trials in Fabry disease)[191], but they are now insufficient for regulatory approval, making the need for good primary clinical end points that relate directly to patient quality of life more important than ever. Biomarkers are still encouraged as secondary supportive end points and are a highly active area of research in many of these disorders. Gaucher disease currently has the most approved therapies, and this is no doubt linked to the fact that the clinical end points are fully validated, easy to measure and may be reached within 12 months of the initiation of therapy[9,186,192]. Clinical end points for CNS disease are much more challenging, as typically we do not know which neurological symptoms reflect neuronal dysfunction versus neuronal loss. Frequently, informed guesswork based on animal model data guides the choice of clinical end points, and thus this process is still far from a precise science. Indeed, most orphan diseases have no validated clinical end points except in a small number of diseases where trials have led to regulatory approval.

The commercial element

Several large companies dominate the ERT field with an established role in this commercial sector. The non-ERT therapies, however, include a fairly large number of smaller commercial enterprises, including start-ups, that have the academic expertise needed to work in a highly specialized and challenging environment. Having a good lead compound or biologic is clearly a prerequisite for success, but many challenges still have to be overcome in order to reach the goal of a marketed therapy. The small companies that commit to and successfully operate in this rapidly evolving space understand the complexity inherent in LSDs early in their development path. However, an increasing number of larger, established companies with no history in the field of LSD treatment are viewing these diseases as a route to get products into common neurodegenerative disease markets by trialling them first in LSDs. This is with a view to orphan incentives and the perception that this will be a quicker path to market. It will be interesting to see how many products from the larger pharmaceutical players make it to common disease markets via this route.

Polypharmacology
Treating a disease using a combination of therapies to maximize clinical benefit.

Drug repurposing

Repurposing of drugs is also highly relevant to the LSD field (for example, the substrate-lowering drug genistein and the small-molecule chaperones ambroxol (Gaucher disease type 3) and pyrimethamine (GM2 gangliosidoses)) (TABLE 2), but these are not the preferred option for the majority of pharmaceutical companies, despite some regulatory incentives. Non-profit organizations such as Findacure are developing innovative platforms to encourage drug repurposing for rare diseases and are looking at health care providers to provide 'social impact bonds' based on the money saved by the use of such drugs. This is very much a 'watch-this-space' area that has the potential to change the way drug repurposing is viewed and is an excellent example of the charitable or non-profit sector driving an innovative agenda for change for the benefit of patients.

Pricing

The vexed issue of pricing is relevant to all rare disease therapeutic products[9]. For CNS diseases, there will likely not be a single drug that is a major disease modifier, and combination therapy will provide the greatest clinical benefit in the future[173,193]. There are serious issues as to how any health-care system can sustain the costs associated with the use of multiple high-price drugs in these chronic diseases, in which lifespan will be extended. From a global perspective, many patients with LSDs live in countries where, unfortunately, they will not be diagnosed and even if they were, they would not have access to treatment due to prohibitive costs. In fact, even in affluent countries, the true health-care economics of LSDs has yet to be fully analysed, and the balance between improved quality of life and drug costs remains a constant battleground for health-care providers, governments and patients alike. Another issue pertinent to CNS diseases is that the cost of treatment is not simply the cost of the specific therapeutic agent. Direct CNS delivery of some products inevitably moves treatment away from the typical home setting (the norm for small molecules and some intravenous ERTs) into the hospital, where each patient may require direct or device-mediated delivery of products to the CNS, typically every two weeks. Who funds these hospital-associated costs is less clear and will vary by health-care system. In the longer term, it will be important to find alternative, minimally invasive delivery methods to treat CNS diseases. Regulatory approval is not the final hurdle that has to be overcome in order to bring a drug to market. Pricing negotiations between manufacturers and the national body that regulates market access can cause a considerable delay in bringing a product into routine use with reimbursement and has to be factored into development timelines.

Outlook

We have seen unprecedented progress in developing new disease-modifying treatments for LSDs over the past 20 years. This is in contrast to the situation with many common neurodegenerative diseases that still lack effective therapies, despite having received much greater research investment over many years. The advances

made in LSD therapy have led to an expansion in both the number and size of companies committed to this area. One exciting development is the diversification away from biologic therapies into innovative small-molecule platforms, with two approved SRTs and the first chaperone therapy approved in 2016. Gene-targeted approaches will no doubt rapidly follow. The challenges ahead are numerous and involve the diagnosis of patients sufficiently early in their disease course for treatments to be maximally effective, having a good knowledge of the natural history of the disease, being able to design pivotal trials through identifying and selecting appropriate clinical end points that respond within a 1–2 year time window and finally pricing drugs in a sustainable way (BOX 2). This is not a commercial space for the faint-hearted, but the unique partnerships between academic, commercial and patient organizations are changing patient lives for the better.

The challenges and successes of therapeutic development for LSDs may serve to inform the treatment of other rare diseases. There can be little doubt that LSD research will also shed light on common diseases of ageing, a further illustration of why studying and treating rare diseases is so important for society at large.

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