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 a LSD - enzyme replacement therapy (ERT) for type 1 Gaucher disease - coupled with regulatory incentives, rapidly catalyzed 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.

Introduction

Lysosomal storage disorders are a family of over seventy rare monogenic diseases that typically present in infancy or childhood and collectively affect 1:5000 live births [1]. However, adult onset forms also occur and are frequently misdiagnosed, so 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 material(s) differs 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(s) is stored, with membrane protein defects the pattern of storage can be more 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 an incomplete knowledge of how lysosomal dysfunction triggers the complex cellular pathogenic cascades that occur in LSDs that cause cell dysfunction and ultimately cell death [7]. Approximately seventy percent of LSDs present as progressive neurodegenerative diseases, highlighting how vulnerable the central nervous system 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, which has significant implications for the development of effective therapies as multiple compartments of the body may require correction/ effective treatment. The availability of authentic 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, were not historically the focus of pharmaceutical industry interest. However, paradoxically LSDs are currently a burgeoning translational field with multiple approved products in routine clinical use and intense academic and commercial activity innovating 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 a LSD, enzyme replacement therapy for type 1 Gaucher Disease [10]. 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 cases at birth and instigate treatment rapidly, should a therapy be available [11, 12]. Early diagnosis of the first affected case in a family also provides the parents reproductive options to prevent other cases being born 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 significant risk of branding a healthy infant with an LSD diagnosis that may never manifest clinically in the individuals lifetime [13].

This review will provide an overview of LSDs and will assess 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 either approved, in clinical trials or where advanced pre-clinical proof of concept has been demonstrated will be discussed.

The Lysosome

The lysosome is an acidic organelle that serves as the major catabolic and recycling center 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 transcription factor E3 (TFE3)[20]. The wider lysosomal system is now appreciated to play a central role in general energy metabolism and the body's response 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 homeostasis. This includes the maintenance of acidic pH and exporting metabolites generated in the lysosome to facilitate their utilization by other organelles/compartments in various aspects of cellular metabolism [22, 23]. These membrane proteins (e.g. 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 so are important for both cellular homeostasis and combatting infection [25]. They also form contact sites with other organelles (e.g. mitochondria and ER) where exchange of ions, lipids and other molecules takes place [26, 27]. This is an area that requires greater research as it will no doubt yield major insights into lysosomal cross talk 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 twenty years, many additional functions of the lysosome have been identified, including nutrient sensing, lysosomal cell death pathways, plasma membrane repair and calcium signaling [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 signaling 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 lysosomal storage diseases [6].

Lysosomal Storage Diseases

Lysosomal storage diseases (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 (e.g. Fabry disease and MPSII (Hunter Syndrome))[1]. Although individually rare (orphan or ultra-orphan), they collectively affect 1:5000 live births and most commonly present as pediatric neurodegenerative diseases [1]. Peripheral tissues/organs can also be affected so these diseases can frequently be multi-system disorders. In isolated human populations and those with high consanguinity rates, their frequency can be much higher. Some at risk populations have introduced a number of successful preventive programs [33] [34] that will be 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 involved in delivering enzymes to the lysosome [37], proteins assisting lysosomal hydrolases to interact with lipid substrates (activator proteins)[38] or proteins exporting cargos 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]. The clinical descriptions of many of these diseases were made over a century ago and in the twentieth century they began to be classified based on the biochemical nature of the storage material and the genes responsible 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 (e.g. enzyme deficiency, transport defect etc.) (**Table 1**). This latter classification system is particularly useful when considering the development of disease modifying therapies [41].

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(s) in a patient leads to their individual rate of disease progression and precise clinical manifestations. Most patients are compound heterozygotes (i.e. they inherit a different mutation in the same gene from each parent) and it is not uncommon for siblings (including twins) that harbor identical mutations to display discordance [42, 43]. A greater understanding of modifier genes, epigenetic modifiers, infectious disease and environmental/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. Therapies developed for LSDs may thus have unanticipated utility beyond the LSD field [44, 45]. Most notably, being a carrier for a Gaucher disease causing mutation confers the highest genetic risk factor for developing Parkinson's disease [45-49].

The LSDs have a major advantage over more common neurodegenerative disease fields in that there are a large number of authentic 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 [50, 51]. This is probably one of the most significant 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 can be due to a number of factors, including the differential biochemistry of distinct cell types, differential turnover rates of substrates, catabolic enzyme redundancy, adaptive changes to counteract the primary defect and whether cells are regenerative or terminally differentiated. In order to treat a 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 will be discussed here. Most affected individuals appear normal at birth and dysmorphia is generally confined to LSDs that affect the extracellular matrix and bone, such as the mucopolysaccharidoses [52]. Suspicion of a LSD is usually triggered by evidence of visceral disease (e.g. hepatosplenomegally in Gaucher and Niemann-Pick B, acute post-natal liver disease in Niemann-Pick type C) or failure to achieve developmental milestones due to the effects of storage in the CNS (e.g. Tay-Sachs disease)[53].

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 etiology may be different in terms of the pathogenic mechanism causing the seizures, but the seizures themselves are not 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 pharmacopeia is therefore the bedrock of current clinical management (palliative pharmacotherapy) for most LSD patients and should not be overlooked when thinking about developing more specific disease modifiers, as management of symptoms already makes an enormous contribution to quality of life for patients and their families [54].

The Diagnostic Odyssey

For most LSD patients 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 foremost in a company's mind when deciding which disease to target (**Box 1**). Diagnosis, therapy, access to therapy and healthcare economics are all confounders in the journey from idea to product and no one should be under any illusions that this is anything other than complex and challenging.

Diagnosis, Prevention and Screening

The diagnosis of an LSD is a totally 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. Diagnostic tests are currently based on various approaches, including measuring lysosomal enzyme levels, cellular assays and mutation analysis with the trend being greater emphasis on molecular diagnostics [55]. Diagnostic delay of several years is unfortunately very common and frequently other children are born to parents before the diagnosis of the first presenting child is made (the index case) [56]. The reasons for diagnostic delay are multifactorial but often result from the result of a lack of clinical awareness due to the rarity of LSDs [57]. 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 counselors [58]. Evaluation of the pros and cons of several diagnostic methods in current use have recently been reviewed [12].

Several health care systems worldwide have developed specialist referral centers where LSD patients can be diagnosed and optimally managed by expert physicians who see a large number of LSD cases 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 those health care systems where patients are seen and kept locally, diagnostic delay is typically longer and clinical management unfortunately not always optimal. The situation with adult onset patients is even less satisfactory with repeat misdiagnoses that can span several decades being the typical experience for most patients. This is because presenting signs in adults frequently resemble more common neuromuscular/neurodegenerative/psychiatric diseases. Also, LSDs are often erroneously viewed as exclusively pediatric disorders; as a consequence many adult onset patients remain undiagnosed and tests to investigate the possibility of a LSD are rarely commissioned outside specialist referral centres. Raising awareness within medical student and health professional training more generally (neurologists, ophthalmologists, hepatologists, hematologists etc.) therefore needs to be a priority [57]. Importantly, rapid diagnosis would allow the parents of an affected child to make informed decision about subsequent pregnancies. One practical aid to prevention would be to introduce newborn screening, with the objective of making the diagnosis of the first affected child, prior to any subsequent pregnancies [56] (**Box 2**). Although this may appear straightforward it is actually surprisingly complex to achieve in practice and raises a number of ethical issues (**Fig. 1**) [11, 59].

Approaches to therapy

In the current era of significant diagnostic delay and a lack of newborn screening/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 that target the apex of this cascade will be most

clinically effective. The various therapeutic approaches are summarized in **Table 2.** Therapies target distinct cell biological processes in different cellular organelles, beyond the lysosome (**Fig. 3)** and so 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 those that target convergent elements of the pathogenic cascade (downstream targets), so 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 LSD patients, but have the major advantage that they have the potential to be the most effective. In contrast, therapies targeting downstream processes have the advantage of being applicable to multiple LSDs potentially but at a disadvantage because they are more likely to be disease modifiers/adjunctive therapies and within the pathogenic cascade they are several steps removed from the primary defect (although there are exceptions which will be discussed, i.e. proteostasis modifiers). Below, therapeutic approaches already in clinical practice or currently being explored for LSDs will be reviewed.

Historical Context For Development of Therapies

It was appreciated early on by Hers and de Duve [60, 61] that most LSDs result from a lysosomal enzyme deficiency and this provides the rationale that underpins the majority of currently approved therapies [35, 62]. The cell biology of lysosomal enzymes is complex, but highly favorable from a therapeutic point of view. Through the pioneering work of Elizabeth Neufeld [63, 64] it was established that lysosomal enzymes mediate a process called cross-correction. Lysosomal enzymes, like other cellular glycoproteins are synthesized in the ER then move through the Golgi where their N-glycans are processed, and often further modified to carry a mannose-6-phosphate (M6P) residue that targets them to the lysosomal system [65, 66]. However, a proportion of the enzyme is released from the cell as a soluble glycoprotein that can be taken up by neighboring cells by binding to surface receptors (e.g. M6P receptor) and subsequently enters the endocytic system and is delivered to the lysosome

where it can function. Pathways independent of M6P also occur [67]; for example LIMP2 is the protein that escorts the lysosomal glucocerebrosidase (GBA) to the lysosome and when deficient causes Gaucher disease [68].

The earliest attempts to treat an LSD caused by an enzyme deficiency employed donor hematopoietic cells administered through the invasive process of bone marrow transplantation (BMT) also termed hematopoietic stem cell transplantation (HSCT). Indeed HSTC as a therapeutic modality is one of the earliest biologic therapies to be put into routine clinical practice [69], with the first report in a Hurler's (MPSI) patient in 1981 [70, 71]. However HSTC has a number of significant limitations. Firstly, it historically required a suitably matched donor to be identified, the recipient had to be immunosuppressed to prevent graft rejection and it is a procedure associated with high levels of morbidity and mortality with very mixed clinical outcomes in LSDs [72]. If the transplant is conducted before the age of 1 year of age clinical outcomes are better [73, 74], which again reinforces the need for early diagnosis. Indeed, this is a major justification for newborn screening for HSTC responsive LSDs (**Fig. 1**). However, despite these limitations, it is an effective disease modifier for some LSDs [72, 73]. As allogeneic BMT is a medical procedure, it is somewhat anomalous, has never undergone the rigors of regulatory approval so falls outside the classical therapeutic development framework. In the modern era, the focus is shifting towards using autologous bone marrow 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 hematopoietic system into an enzyme producing/secreting "factory" (see below). Other cell-based therapies, such as neural stem cell therapies, have shown some efficacy in animal models and have been in clinical trials in a very small number of LSDs [75-77] 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 spring 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 ((**Table 2**)) [78-83] or "replace" the defective protein by administering a recombinant wild type protein into the patient's circulation or deliver it directly to the CNS via a device [84-88].

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/organs, so 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 and for the current generation of patients either means the CNS remains untreated or that 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, we are at a point where we can change the natural history of these diseases through correcting/partially correcting one set of clinical phenotypes but the extended lifespan of the patient allows for the emergence of new symptoms. This raises a number of challenging ethical questions beyond the scope of this article.

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), retroviral or lentiviral vectors [83](**Table 3**). The discovery that AAV9 can be administered intravenously and it can correct the periphery and cross the blood-brain barrier raises the prospect of much less invasive gene therapy delivery in the future [89]. An alternative strategy that requires lower amounts of vector is to perform HSCT *ex vivo* gene therapy by introducing autologous corrected haematopoietic stem cells back into the patient's circulation [83]. Macrophage lineage cells can then migrate from the bone marrow to the CNS and differentiate into microglia and serve as a source of fully functional enzyme. Indeed, this is the basis for how HSTC without gene correction is beneficial in some CNS disorders. However, the numbers of cells that migrate into the brain are relatively small so this is not a very efficient process and is of limited clinical efficacy [90].

When it comes 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 (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 diseases with death in infancy/early childhood. On the other hand, patients with a juvenile onset form of the disease present at a later age and live longer, whilst adult-onset patients may not develop clinical signs until well into adulthood and have a relatively normal life expectancy, albeit with a burden of disabling neuromuscular disease. Remarkably, the differential levels of residual enzyme between different ages of clinical onset are actually quite subtle [91]. Therefore, even relatively inefficient gene therapy could generate sufficient wild type, fully functional enzyme to convert severe disease into 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 significant levels of irreversible neuropathology have taken place.

There are currently multiple pre-clinical studies that show benefit of gene therapy in both small and large animal models and some of these have moved in to Phase I /II safety studies in LSD patients (**Table 3**). For example, two forms of neuronal ceroid lipofuscinosis (CLN2 and 6) are in phase I and I/II trials, using AAV-based vectors delivered intra-cranially or via an intrathecal route. There are also multiple phase I/II trials in MPS diseases (MPSII, IIIa and IIIb) using either retroviral *ex vivo* gene correction of HSCT for MPSII or AAV vectors delivered intravenously or intracranially for MPS III (**Table 3**). The fact that some gene therapy vectors are already approved [92] 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/proteins 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 neighboring non-transduced cells, which cannot happen in the case of membrane proteins. However, recent findings in pre-clinical studies have shown that when the lysosomal membrane protein NPC1 (a large 13 trans membrane-pass protein deficient in most cases of Niemann-Pick type C disease) was introduced into NPC1 null mice using AAV vectors, clinically-relevant benefit resulted, suggesting that gene therapy for lysosomal membrane proteins may be a viable approach after all [93]. This is a very active preclinical area of research at the moment and offers some hope to patients suffering from membrane protein deficiencies, which accounts for a significant number of monogenic human diseases. Other approaches to tackle genetic defects directly include stop codon read-through 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 [94], with many more compounds now under evaluation/development and have been reviewed comprehensively in the context of LSDs very recently [95]. To date seventeen proof of concept studies have been conducted *in vitro* and in some murine model studies across multiple LSDs primarily using gentamycin. Screens to identify proprietary molecules have yielded for example PTC124 (ataluren, PTC Therapeutics), which has shown efficacy *in vitro* and in a mouse model of Cln1 and is EMA approved for Duchenne muscular dystrophy [95]. 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 targeting target organs effectively [96, 97]. This rapidly evolving field has been reviewed recently [98].

Enzyme Replacement Therapy (ERT)

The first enzyme replacement therapy (β-glucocerebrosidase) was pioneered for type 1 Gaucher disease by Roscoe Brady and colleagues at the NIH [10, 99]. The first product was placentally derived (Ceredase, Genzyme Corporation) and was FDA approved in 1991 following a small open label clinical trial. It is worth reflecting for a moment on features of this landmark clinical trial. There was no

placebo arm, no complex trial design, but just a very clear clinical outcome using clinical end points that could be measured easily. This included reduced liver and spleen volumes and improved hematological parameters (e.g. improvement/correction of anemia and thrombocytopenia). We have yet to see another ERT with such efficacy, hence trials tend to involve greater numbers of enrolled patients, be placebo controlled and often require multiple trials. A good example of the modern ERT trial is acid sphingomyelinase ERT for Niemann-Pick type B. This looks extremely promising [100, 101]. However, the regulators have requested a phase III trial, thereby inevitably delaying patient access to treatment for a number of years. It could be argued that phase III trials are only appropriate for diseases with large patient numbers and approval with post-marketing surveillance would be a good compromise. This issue is regularly debated within the rare disease field but has not been adequately resolved. It in part reflects the fact that the drug approval process does not differentiate between the divergent needs of rare and common diseases and has a single process to deal with these two very different disease sectors.

Returning to ERT for Gaucher disease, the placental enzyme was replaced with recombinant enzyme expressed in CHO cells (Cerezyme). This remarkable translational achievement and its catalysis of the development of other therapies for LSDs have been extensively reviewed following Roscoe Brady's death in 2016 [97, 102-108]. The clinical efficacy and commercial success of ERT for Gaucher disease catalyzed the development of ERT products for other LSDs (4). Furthermore, companies have developed multiple ERTs for Gaucher disease alone, with three products on the market along with bio-similars [109]. Several new ERTs for other LSDs are currently in clinical trials, including some that are administered directly to the CNS including tripeptidyl peptidase for treating late infantile NCL (intra-cerebroventricular 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 dependent upon the stage in the disease course when treatment is initiated. Early intervention is key as the disease can then be positively modified prior to the development of irreversible pathology [107]. One important consequence of the commercial activity in LSDs is that it has driven the process of improving rates of diagnosis and identifying

patients as early as possible to rapidly initiate treatment [56]. The limitations of ERT include high cost preventing access; invasive routes of delivery (most typically intravenous); infusion reactions owing to hypersensitivity in some patients; and lack of penetrance of the enzyme to key pathological sites (e.g. brain and bone)[9]. Nonetheless, there is no doubt that, as a class of biologic therapies for LSDs, ERT has very significantly improved quality of life for many patients suffering from several LSDs and will continue to do so [99, 107, 110].

Disease Specific Therapies for LSDs: small molecules

Because ERT has a number of limitations, other approaches have been sought to enhance 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 a protein product that fails to pass the ER quality control machinery so never reaches the lysosome and is 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, they will only work in those patients with some residual enzyme function and not all mutations are amenable to this approach. For each enzyme a different chemistry of chaperone is needed, so 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 counter intuitive, but is based on the finding that if a mutant, unstable enzyme binds a small molecule in its active site (e.g. a substrate mimetic) the active site is stabilized and remains stable once the small molecule has dissociated [111, 112]. This approach is therefore dependent on the fact that the off rate for the inhibitor favors dissociation after the enzyme is stabilized as otherwise the

enzyme levels in the patient would be further reduced, not enhanced, due to sustained inhibition. Sub-inhibitory concentrations of these drugs are also used to favor enzyme enhancement. A major advantage of this approach is the wealth of small active site inhibitors known, many of which are imino sugar drugs [113]. Inhibiting hydrolases has been a very active area of research for many decades and so chaperones are relatively straightforward to identify in conventional biochemical screens [113-121]. 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, molecular chaperones have entered clinical trials without this typical step in pre-clinical development that usually requires efficacy to be demonstrated in an authentic animal model. The problem with the current generation of compounds is that it is quite challenging to devise a dosing regimen that favors enhancement of enzyme function relative to inhibition. However, the recent approval of the active site inhibitor migalastat (2016)[122-124] by the EMA for the treatment of Fabry disease is a landmark for this approach and uses an active site inhibitor (Amicus Therapeutics)[125]. This imino sugar drug involves a treatment regimen of every other day dosing in order to balance enzyme inhibition/stabilization with the resulting enhanced enzyme activity. Another chaperone showing promise is the repurposed drug Ambroxol for treating neuronopathic type 3 Gaucher disease that is in investigator led clinical studies at the present time [118, 126-130].

To overcome the limitation of active site inhibitors, a new generation of chaperones is being developed that are allosteric enhancers [113, 131, 132]. Here, the small molecule binds away from the active site but induces a conformational change/stabilization that enhances enzyme activity or extends half-life. A promising non-inhibitory compound to emerge from a 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 iPSCs from patients with Gaucher and Parkinsonism [133]. 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 and mutation specific so require careful testing of patient cells with a given mutation(s), to assess their individual suitability for this approach [113]. This is an area of LSD drug discovery that therefore encompasses personalized medicine [134].

Non-Disease Specific therapies for LSDs: small molecules

Substrate reduction therapies

The first small molecule therapies to be approved for LSDs were substrate reduction therapy (SRT) drugs [135] (**Fig.3**). SRT does not target the mutant enzyme, but instead prevents the build up of the substrate(s)[136, 137]. 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 Norman Radin and was "reduced to practice" in the glycosphingolipid storage diseases [135, 138]. With the exception of galactosylceramide and its derivatives present in myelin, all other glycosphingolipids (GSLs) are synthesized through a common biosynthetic pathway that begins with the transfer of glucose to ceramide to form glucosylceramide (GlcCer)[139]. 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 catalyzed by glucosylceramide synthase (GCS), and this transferase is the target for the two currently approved drugs, miglustat (Zavesca, miglustat)(Actelion) and (Cerdelga, eliglustat)(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 CGS inhibitor relative to eliglustat, which is a longer chain ceramide mimetic. Miglustat crosses the blood brain-barrier to some extent, whereas eliglustat does not [140]. Miglustat inhibits gastrointestinal tract disaccharidases, so its main side effect is osmotic diarrhea [141]. Miglustat was first approved in 2002/3 as a second line treatment for type 1 Gaucher disease (EMA and FDA) and in 2009 for

Niemann-Pick type C (EMA). Eliglustat was more recently approved (2014) as a first line oral therapy for type 1 Gaucher disease (FDA/EMA)(**Table 2**) and requires patient genotyping to ascertain their Cyp2D6 status [140]. Other drugs that are metabolized by Cyp2D6 may be contraindicated [142, 143].

Miglustat was the first oral small molecule therapeutic and both miglustat and eliglustat offer type 1 Gaucher patients 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 in Fabry disease [144-146]. Genzyme are developing CNS penetrant SRT drugs with a view to treating CNS disease in the glycosphingolipid storage diseases. Currently Ibiglustat ((Genz-682452) is in phase II trials for Fabry (NCT02226084), Gaucher (NCT02843035) and Parkinson's (NCT02906020).

SRT for other LSDs is currently very limited although genistein is currently in clinical trials for Mucopolysaccharidosis type IIIB (Sanfilippo syndrome, alpha-N-acetylglucosaminidase deficiency)) [147]. Genistein is an isoflavone abundant in soya and acts as broad-spectrum protein tyrosine kinase inhibitor that acts on EGF and IGF receptors that regulate proteoglycan biosynthesis (proteoglycans are stored in MPS diseases). Genistein also modulates TFEB function adding another dimension to this phytoestrogen's pharmacological properties [148, 149]. A phase III, randomized, placebo controlled trial of high dose Genistein aglycone is fully recruited in Europe in children and adolescents less than 18 years of age with a proven diagnosis of Sanfilippo syndrome (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 [150].

Proteostasis modifiers

Another strategy that involves the use of a small molecule is to enhance the endogenous cellular response to stress and promote up-regulation of the chaperone heat shock protein 70 (HSP70) to promote protein folding [151]. HSP70 was also found unexpectedly 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 also stabilizes acid sphingomyelinase, thereby enhancing its activity by prolonging its half-life. This increases ceramide levels in lysosomal membranes and consequently reduces lysosomal membrane permeability [152]. The first cellular proof of concept for the use of HSP70 for treating a LSD was the discovery that Niemann-Pick disease type A/B cells could be corrected *in vitro* [152]. These studies were then extended to a panel of other LSD derived cell lines, demonstrating broad efficacy [153]. 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 [153] that induces HSP70 expression. Arimoclomol achieves HSP70 induction through stabilizing a transcription factor (activated HSF1), which binds to heat shock elements in the promoter of heat shock inducible genes, including HSP70 [154, 155]. This drug therefore induces HSP70 only in cells that are already stressed and does not induce stress itself, as other HSP70 inducers have been recognised to do [156]. This drug is not restricted in its potential use to LSDs but is also being investigated clinically for Amyotrophic Lateral Sclerosis (ALS) [157]. A clinical trial of arimoclomol in NPC disease is currently in progress (Orphazyme, NCT02612129). This drug has the potential to be used in multiple LSDs [153] as its mechanism of action is not disease specific. Another regulator of proteostasis, the drug celastrol has also been evaluated in Gaucher disease and also enhanced the effects of arimoclomol [158, 159]. However, celastrol is a stress inducer and showed evidence of toxicity in some model systems in which it was tested [160].

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 [161, 162]. Some anti-inflammatory therapies or genetic manipulations have been trialed in animal models and suggest that not only is inflammation an active contributor to pathogenesis [161] but also represents a therapeutic target [163,

164]. For example, synergy was demonstrated in a mouse model of NPC disease when anti-inflammatory drugs were combined with miglustat (SRT) and a calcium modulator curcumin [164]. Clinical trials are needed to determine the extent of disease modification achievable, but could be a promising area of research particularly because it involves drug repurposing using existing therapies that are already on the market for treating chronic inflammatory diseases, thereby speeding the path to translation.

The involvement of the complement system, specifically C5a and C5aR, has recently been implicated in driving inflammation in genetic and pharmacologically induced models of Gaucher disease. 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 more glucosylceramide, the main storage lipid in Gaucher disease. C5a was also found to be elevated in Gaucher disease patient sera in the same study [165]. This raises the question as to whether targeting C5aR may be a future strategy to treat Gaucher disease [165]. 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 [166] but the generality of this finding remains largely unexplored. Anti-glycosphingolipid antibody pathophysiology is complex as it is dependent upon the nature of lipid environment in which the glycosphingolipid epitope is present, an important finding arising from detailed studies in the autoimmune disease, Guillain-Barre syndrome [167-169]. The presentation of glycosphingolipids by CD1d to invariant Natural Killer T cells (iNKT) is another immunological axis potentially involved in immune dysfunction in glycosphingolipid lysosomal storage diseases and is an area of very active research in mouse models and patients [170-173]. The lysosome plays a key role in processing antigens that can be loaded onto CD1d and also contains activator proteins that facilitate the loading of these lipids. As a consequence, lysosomal dysfunction can affect CD1d/iNKT cell biology leading to changes in iNKT cell numbers and function [170]. However, there are significant species differences with CD1d localizing to the lysosome in murine models where as 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 do date it remains unclear how changes in iNKT cell biology may contribute to LSD pathogenesis[170].

Challenges and considerations

The discovery of enzyme cross correction by Liz Neufeld and the pioneering research by Roscoe Brady led to Genzyme launching the first disease specific LSD product on the market in 1992 [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 if any products have achieved the same degree of clinical success. This poses a number of problems in what is now a much more crowded commercial space and where the "low hanging fruit" (i.e. those LSDs without significant 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/ 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 aging population living with multiple, typically chronic clinical conditions, a situation termed multimorbidity [174, 175]. 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, hematological abnormalities (including myeloma) and neurological disease, which at the extreme end (type 2 disease) involves

acute neurodegeneration [176-178]. It may be timely when thinking about how to deal with multimorbidity in the general population 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. Potentially, the specialist referral centers for LSDs in the UK and other European countries would be one model to emulate, as they are highly affective in diagnosing and managing these complex disorders.

Polypharmacology

The drugs developed to date (dominated by biologics i.e. 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 [179]. 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 {Grabrucker, 2016 #7317}. Further more, even effective ERTs do not access all 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 [176]. So the "Holy Grail" will be development of therapies that treat all compartments of the body effectively. We are certainly closer to achieving this in the modern era, but unfortunately this remains an unmet aspiration in terms of 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, each therapeutic agent targeting unique aspects of the pathogenic cascade [136](**Fig. 2**).

Clinical endpoints

In the past, biomarkers were used as primary endpoints in some clinical trials (e.g. biochemical measurement of the stored glycosphingolipid, Gb3, in Fabry ERT trials)[180], which are now not permitted within the current regulatory environment, making the need for good primary clinical endpoints that relate

directly to patient quality of life more important than ever. Gaucher disease currently has the most approved therapies and this is no doubt linked to the fact that the clinical endpoints are fully validated, easy to measure and respond within 12 months of the initiation of therapy [9, 177, 181]. Clinical endpoints for CNS disease are much more challenging as typically we do not know which neurological symptoms reflect neuronal dysfunction versus neuronal loss. It frequently comes down to informed guesswork based on animal model data that guides the choice of clinical endpoints so it is still far from a precise science.

The Commercial Element

Several large companies dominate the ERT field with an established role within this commercial sector. The non-ERT therapies however include a relatively large number of much smaller commercial enterprises, including start-ups, who have the academic expertise needed to work in a highly specialized and challenging environment. Having a good lead compound/biologic is clearly a pre-requisite 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 on 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 trialing them first in LSDs. This is with a view to Orphan Incentives and a perceived view that this will be a quicker path to market. It will be interesting to see how many such products from the bigger pharmaceutical players make it to common disease markets via this route [182, 183].

Drug repurposing

Repurposing of drugs is also highly relevant to the LSD field (e.g. the substrate lowering drug genistein, 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

(http://www.findacure.org.uk) are developing innovative platforms to encourage drug repurposing for rare diseases and are looking at health care providers to give "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/not for profit sector driving an innovative agenda for change for the benefit of patients.

Pricing

There is the vexed issue of pricing that is relevant to all rare disease therapeutic products [9]. For CNS diseases there is very likely not going to be a single drug that is a major disease modifier and combination therapy will provide the greatest clinical benefit in the future [182, 183]. There are serious issues as to how any health care system can sustain the costs associated with using multiple high price drugs in these chronic diseases, in which life span will be extended. From a global perspective, many LSD patients live in countries where unfortunately they will not be diagnosed and even if they were, 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 analyzed 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 for CNS diseases is that it 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 intravenous ERT) into the hospital where each patient may necessitate direct or device-mediated delivery of products to the CNS typically every two weeks; who will cover these additional medical costs remains unclear. In the longer term it will be important to find minimally invasive delivery methods for treating the CNS to remove from patients and their families the considerable burden of frequent hospital-based administration of therapeutics. Regulatory approval is not the final hurdle that has to be overcome in order to bring a drug to market. Pricing negotiations between manufacturer and the national body that regulates

market access can cause considerable delay in bringing a product into routine use with reimbursement and has to be factored into development time lines.

Outlook

We have seen unprecedented progress in developing new disease modifying treatments for LSDs over the past twenty years. This is in contrast to the situation with many common neurodegenerative diseases that still lack effective therapies, despite them 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 of the exciting developments is the diversification away from biologic therapies into innovative small molecule platforms, with two approved SRTs and the first chaperone therapy approved in 2016. The gene-targeted approaches will no doubt rapidly follow. The other 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 to price drugs in a sustainable way (**Box. 1**).

From Rare to Common

Monogenic diseases are often referred to as "simple" genetic diseases and at one level they are. However, everything downstream of the defective gene, in terms of pathogenesis, clinical heterogeneity, diagnosis, prevention, defining responsive clinical intervention points, trial design, regulatory framework and ultimately pricing and reimbursement is anything but simple. This is not a commercial space for the feint hearted, but the unique partnerships between the academic, commercial and patient organizations are changing patient lives for the better. Additionally, the challenges and successes of therapeutic development for LSDs may also 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

aging, a further illustration of why studying and treating rare diseases is so important for society at large.

Conflicts of interest

FP is a consultant to Actelion, E3Bio and Orphazyme, and a co-founder and consultant to IntraBio.

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