Treating lysosomal storage diseases with pharmacological chaperones: from concept to clinics

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Keywords: lysosomal storage diseases; pharmacological chaperones; enzyme replacement therapy; pharmacological chaperone therapy; proteostasis regulators

DOI 10.1002/emmm.200900036

Received June 10, 2009 / Revised June 29, 2009 / Accepted June 30, 2009

Lysosomal storage diseases (LSDs) are a group of genetic disorders due to defects in any aspect of lysosomal biology. During the past two decades, different approaches have been introduced for the treatment of these conditions. Among them, enzyme replacement therapy (ERT) represented a major advance and is used successfully in the treatment of some of these disorders. However, ERT has limitations such as insufficient biodistribution of recombinant enzymes and high costs. An emerging strategy for the treatment of LSDs is pharmacological chaperone therapy (PCT), based on the use of chaperone molecules that assist the folding of mutated enzymes and improve their stability and lysosomal trafficking. After proof-of-concept studies, PCT is now being translated into clinical applications for Fabry, Gaucher and Pompe disease. This approach, however, can only be applied to patients carrying chaperone-responsive mutations. The recent demonstration of a synergistic effect of chaperones and ERT expands the applications of PCT and prompts a re-evaluation of their therapeutic use and potential. This review discusses the strengths and drawbacks of the potential therapies available for LSDs and proposes that future research should be directed towards the development of treatment protocols based on the combination of different therapies to improve the clinical outcome of LSD patients.

Introduction

Lysosomal storage diseases (LSDs) are a group of genetic disorders caused by defects of activity, post-translational modifications and trafficking of soluble lysosomal hydrolases, integral membrane proteins and transporters (Futerman & van Meer, 2004). These defects cause impaired intracellular turnover and disposal of a variety of substrates, including sphingolipids, glycosaminoglycans, glycoproteins and glycogen. The accumulation in the endosome/lysosome of these substrates elicits complex, and still only partially understood, secondary biochemical and cellular events that ultimately lead to cell and tissue damage (Ballabio & Gieselmann, 2009; Futermann & van Meer, 2004).

More than 40 LSDs are known and are traditionally classified according to the chemical properties of the accumulated compound. Although each of them is rare, their overall prevalence is estimated as 1 in 8000 live births (Meikle et al, 1999), and they represent a heavy burden in terms of patients' health, social and economical costs.

LSDs are characterized by involvement of multiple tissues and organs. Therefore, LSD phenotypes are complex, with the variable association of visceral, skeletal and neurological manifestations. Among the clinical manifestations, those related to central nervous system (CNS) involvement, often with progressive neurodegeneration and cognitive impairment, are highly debilitating.

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The complexity of LSD phenotypes and pathophysiology makes therapeutic interventions for LSDs particularly challenging. Therapies should be directed towards correction of pathology and function in all affected tissues to restore health, or to ameliorate patients' quality of life. Although different therapeutic options have been introduced over the past two decades (Fig 1), it is now becoming clear that these approaches have limitations and important issues remain unsolved. In addition, each of the diverse approaches currently available have strict indications and criteria of applicability for individual LSDs, or even for subsets of patients within each disease, and, in principle, none of them have the potential to treat all diseases (Table 1).

For these reasons, current efforts are being directed towards exploring alternative therapeutic approaches for LSDs based on innovative strategies.

Current approaches for LSDs: successes and failures

Among the different therapeutic approaches for LSDs, substrate reduction therapy (SRT) aims to reduce the rate of substrate synthesis, thereby restoring the equilibrium between substrate synthesis and degradation. The strategy is used in the treatment of type 1 Gaucher disease since 2003 and is based on the use of small molecule inhibitors of glycosyltransferases (reviewed in Platt & Jeyakumar, 2008). It is effective in improving or stabilizing biochemical, haematologic and bone markers of disease (Pastores et al, 2005). Clinical studies are in progress for the evaluation of this strategy in Niemann–Pick disease type C, a defect of intracellular cholesterol trafficking in which glycosphingolipid storage plays a pathogenic role in brain disease (Patterson et al, 2007), whereas pre-clinical studies have been

Glossary

Blood–brain barrier (BBB)
A selectively permeable barrier between the capillaries and the brain that acts as a filter preventing many substances from entering the CNS.

Chaperones
Innate proteins (molecular chaperones) or small molecule drugs (pharmacological chaperones) that assist protein folding and maintenance of native conformation.

Endosome/lysosome
Vesicular organelles involved in the trafficking and sorting of endocytosed cargo molecules, and in the degradation and recycling, by lysosomal hydrolases, of complex substrates.

Fabry disease
An X-linked multisystem disease caused by the deficiency of the lysosomal hydrolase GLA, characterized by storage in blood vessel endothelia, and cardiac, renal, cutaneous and ocular manifestations.

Gaucher disease
An LSD caused by GBA deficiency and characterized by sphingolipid storage in reticulo-endothelial cells. Gaucher disease's phenotype is characterized by visceral, haematological and skeletal manifestations. In 'neuronopathic' Gaucher phenotypes (types II and III), progressive neurological involvement is an additional and debilitating manifestation.

G_{M1}Gangliosidosis
An LSD caused by β-galactosidase deficiency. The phenotype of the disease is characterized by visceral, ocular and neurological manifestations.

Heat–shock response
Up-regulation of functionally related proteins in response to elevated temperature or stress.

Krabbe disease
A leukodystrophy caused by the deficiency of the lysosomal hydrolase β-galactocerebrosidase and characterized by a fatal neurodegenerative clinical course.

Lysosomal storage diseases (LSDs)
A group of more than 40 genetic disorders. The majority of these diseases are caused by the defective activity of soluble lysosomal hydrolases, which are involved in the degradation of complex molecules. Other defects are caused by defective function of integral membrane proteins and transporters.

Mannosidosis
A lysosomal storage disorder of glycoprotein catabolism caused by the deficiency of α-mannosidase. The phenotype of mannosidosis is characterized by skeletal and visceral symptoms, and by progressive neurological deterioration.

Metachromatic leukodystrophy
A LSD due to arylsulphatase A deficiency and characterized by progressive neurodegeneration.

Mucopolysaccharidoses
A group of LSDs, caused by defects of lysosomal hydrolases involved in glycosaminoglycan degradation. The clinical phenotype of these disorders is characterized by the variable association of neurological involvement, visceral, skeletal and ocular manifestations.

Niemann–Pick disease type C
A defect of intracellular cholesterol trafficking due to mutations of either NPC1 (95%) or NPC2 (5%) genes, and characterized by progressive visceral and neurological manifestations.

Pompe disease
A metabolic myopathy caused by the deficiency of the lysosomal hydrolase GAA. Pompe disease's phenotype is highly variable and ranges from severe infantile presentations with cardiomyopathy, hypotonia and early lethality, to attenuated late-onset forms with absent or minimal cardiac involvement.

Private mutation
A rare mutation, usually found in a single family or small population.

Proteostasis
An innate cellular network of pathways that control the equilibrium of protein synthesis, folding, trafficking, aggregation, disaggregation and degradation

Tay–Sachs disease (G_{M2} Gangliosidosis)
An LSD caused by β-hexosaminidase deficiency. The phenotype of the disease is characterized by ocular and severe neurological manifestations.

Unfolded protein response (UPR)
A cellular stress response to the accumulation of misfolded proteins, resulting in coordinated activation and transcription of genes aimed at increasing the production of molecular chaperones.

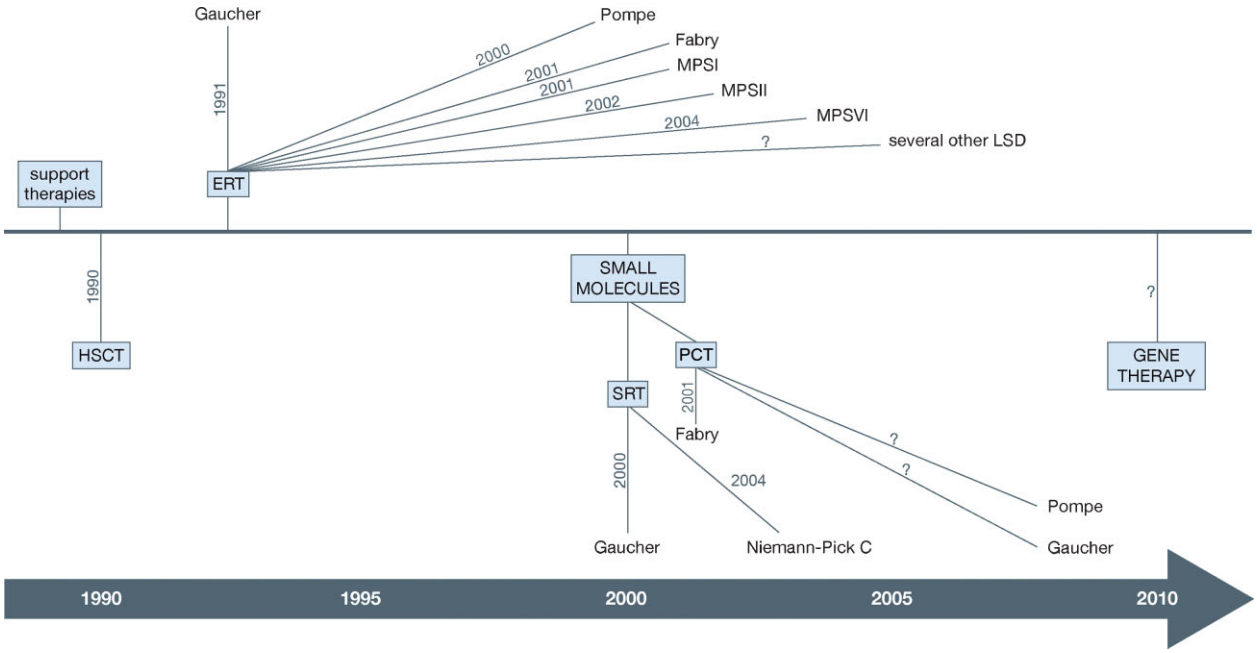

Figure 1. Therapeutic approaches for LSDs. Till the end of the 1980s, only support therapies were available for LSD patients. During the past two decades, different therapeutic approaches have been introduced, including HSCT, ERT, PCT and SRT. Each of these approaches has been applied to different diseases. HSCT has been applied to several LSDs, not indicated in the figure (for a review, see Orchard et al, 2007). The year of the first reports on clinical applications of each therapy is indicated. ERT is now under development for several LSDs. EET has been translated for Fabry disease. Two studies on PCT for Gaucher and Pompe disease are in progress, but the results are not yet published. Although gene therapy pilot studies have been conducted (Worgall et al, 2008) or are in preparation, it is currently difficult to predict when their clinical translation will take place in this area.

published for mucopolysaccharidoses (MPS) (Piotrowska et al, 2006; Roberts et al, 2007) and a few other LSDs (Andersson et al, 2004; Elliot-Smith et al, 2008).

The majority of therapeutic approaches for LSDs are aimed at correcting the loss of function of the mutated enzyme by increasing the cell and tissue levels of a functional protein. Among them, haematopoietic stem cell transplantation (HSCT) was the first to be introduced in the treatment of LSDs. This strategy relies on the use of healthy haematopoietic stem cells from a donor and is based on the effects of repopulation of specific tissues by the donor's cells, and on the ability of these cells to secrete functional lysosomal enzymes and correct the

Table 1. Advantages and limitations of therapies for LSDs

	Advantages	Limitations
HSCT	• Sustained correction after a single procedure • Cross-correction of host's cells by secreted enzymes	• Procedure-related risks and mortality • Not effective in some diseases • Time required to identify compatible donors • Poor engraftment in tissues like bone, cartilage and heart
ERT	• Long-term experience and documented efficacy in thousands of patients treated (*e.g.* Gaucher disease) • Registries available to document natural history of disease and efficacy	• Poor distribution of recombinant enzymes in specific tissues • Inability of recombinant enzyme to cross the BBB; • Frequent infusions required, with high impact on quality of life • High costs
SRT	• Oral administration • Little impact on quality of life	• Limited clinical experience (with the exception of Gaucher disease) • Long-term adverse effects unknown
PCT	• Better biodistribution of therapeutic agents • Possibility to target neurodegeneration in LSDs • Oral administration • Little impact on quality of life	• Limited clinical experience • Long-term adverse effects unknown • Only patients with specific 'responsive' mutations amenable to treatment
Gene therapy	• Sustained correction after a single procedure • Cross-correction by enzymes secreted by 'factory' organs	• Very limited clinical experience, still under development

host's deficiencies. Although burdened by a significant level of procedure-related mortality and by insufficient engraftment in tissues such as bone or heart, HSCT is indicated for the treatment of MPS I and has beneficial effect on the visceral manifestations of MPS VI, in pre-symptomatic or late-onset Krabbe disease, and in the attenuated forms of metachromatic leukodystrophy (Orchard et al, 2007).

Gene therapy, is aimed at restoring enzyme activity by delivering the wild type copy of the defective gene into the recipient's cells. Gene therapy is under study for a variety of diseases, mostly in pre-clinical studies performed in animal models recapitulating the LSDs, phenotypes. Multiple viral vectors have been used to accomplish *in vivo* gene transfer, such as herpesviruses, lentiviruses, adeno-associated viruses (AAV), adenoviruses (Ad) and others (Sands & Davidson, 2006).

This review focuses particularly on enzyme replacement therapy (ERT) and on pharmacological chaperone therapy (PCT). The former represented a major advance in the treatment of LSDs and is currently the standard of care for the most prevalent of these disorders, such as Gaucher, Fabry and Pompe disease and MPS I, II and VI (Table 2) (Brady, 2006). PCT on the other hand, is the newest proposed therapeutic strategy and is in active and rapid development (Fan & Ishii, 2007).

The rationale of ERT evolved from the identification of the molecular mechanisms implicated in the sorting of newly synthesized and secreted enzymes. ERT is based on the concept that recombinant lysosomal hydrolases, mostly enzyme precursors, can be internalized by cells and tissues through the mannose or mannose-6-phosphate receptor (MPR) pathways and are ultimately delivered to lysosomes, where they are activated and replace the function of the defective hydrolases (Brady, 2006).

Further impulse to the development of ERT derived from the availability of technologies allowing large-scale production, purification and manipulation of the oligosaccharide moieties of recombinant proteins to improve cell and tissue targeting, and from the Orphan Drug legislation, that encouraged biotech companies to invest in the treatments for rare diseases previously ignored for their limited profitability.

After several years and after thousands of patients have been treated, the success of ERT has been clearly documented in Gaucher disease, the first disease targeted by this approach with an extractive β-glucocerebrosidase (GBA) (Barton et al, 1991 registries). This stimulated investigators and companies to develop new recombinant enzymes and to extend ERT to other LSDs (Table 1 and Fig 1).

Despite considerable success in treating some of these diseases, ERT clearly has limitations. A major problem derives from enzyme biodistribution. Recombinant enzymes are large molecules that do not freely diffuse across membranes and depend on the mannose or MPR pathways for delivery to lysosomes. For example, in MPS I, II and VI, distribution of recombinant enzymes and correction of pathology is inefficient in tissues such as bone, cartilage and heart (Wraith, 2006).

Of even greater clinical relevance is the inability of recombinant enzymes to cross the blood–brain barrier (BBB). Many LSDs are characterized by CNS pathology and progressive neurodegeneration, and obtaining corrective enzyme levels in the brain is a major therapeutic goal. Attempts to address this issue have been made, based on different strategies. For example, chemical modification of β-glucuronidase abolished mannose and MPR-mediated uptake of the enzyme, and resulted in sustained enzyme plasma levels and increased delivery to brain cells by an unknown pathway (Grubb et al, 2008). Other approaches are either based on receptor-mediated penetration of BBB, such as by coupling recombinant enzymes with transferrin and using transferrin receptor-mediated endocytosis for transport across the BBB (Osborn et al, 2008), or on invasive procedures, like intratechal ERT (Munoz-Rojas et al, 2008). For all these studies, clinical translation does not appear imminent.

In Pompe disease, a metabolic myopathy caused by defective activity of acid α-glucosidase (GAA), insufficient targeting of the therapeutic enzyme to the major sites of storage and pathology, *i.e.* heart and muscle, has also been observed. In particular, correction of skeletal muscle disease by ERT with recombinant human GAA (rhGAA) appears exceptionally challenging (van der Ploeg & Reuser, 2008). Unfortunately, rhGAA is preferen-

Table 2. ERT in LSDs

Disease	Defective enzyme/gene	First report(s)	Recombinant enzyme preparation(s)	Status	Average cost per patient per year[(1)] (USD)	Other therapeutic approaches available
Gaucher disease	GBA	Barton et al, 1990	Imiglucerase	Approved	90,000–720,000	SRT, PCT
Fabry disease	GLA	Eng et al, 2001; Schiffmann et al, 2001a, 2001b	Agalsidase alpha; Agalsidase beta	Approved	156,000–215,000	PCT
Pompe disease	GAA	van den Hout et al, 2000	Aglucosidase alpha	Approved	476,000	PCT
MPS I	IDUA	Kakkis et al, 2001	Laronidase	Approved	260,000–340,000	—
MPS II	IDS	Muenzer et al, 2002	Idursulphase	Approved	657,000	—
MPS VI	ARSB	Harmatz et al, 2004	Galsulphase	Approved	377,000	—
Niemann–Pick B	ASM	—	—	Trial phase I	—	—
Mannosidosis	MAN2B1	—	—	Pre-clinical	—	—
Metachromatic leukodystrophy	ARSA	—	—	Trial phase I–II	—	—
Krabbe disease	GALC	—	—	Pre-clinical	—	—

[(1)]Beutler et al, 2006; Wraith et al, 2006.

tially taken up by the liver and only a minor fraction of the enzyme is delivered to skeletal muscle, which expresses low levels of the MPR. To increase the targeting of the rhGAA to the muscle, a glycoengineered GAA with improved affinity for the MPR (Zhu et al, 2009) has been developed, and resulted in improved enzyme delivery into muscles, higher clearance of glycogen and improvement of muscular function and strength in the mouse model of the disease.

The inefficient biodistribution of the recombinant enzymes is however not the only limitation of ERT. Aspects related to the pathophysiology of LSDs and the secondary cellular abnormalities elicited by storage also affect clinical outcomes and are not addressed by ERT. In fact, they may even impair ERT efficacy. Again, Pompe disease is a paradigm of these problems. In an animal model, Pompe disease autophagic build-up has been observed in muscle cells, resulting in sequestration of the rhGAA in the autophagic compartment (Fukuda et al, 2006). In addition, disruption of the MPR pathway and reduced efficacy of the recombinant enzyme has been observed in patient-derived fibroblasts (Cardone et al, 2008).

Adverse reactions or attenuated therapeutic efficacy of ERT may be the consequence of the immune response to recombinant enzymes. Also, the frequent intravenous infusions to obtain sustained corrective enzyme levels and the need for permanent intravenous devices impose a burden on patients' quality of life.

Finally, economical aspects should not be ignored. Initial investments in research and costs related to the production of large amounts of good manufacturing practice (GMP) recombinant enzymes, contribute to keep the prices of ERT extremely high. The treatment for a single patient may cost as much as several hundred thousand US dollars (Beutler, 2006), thus making ERT one of the most expensive therapeutic strategies currently in use (Table 2). Such high costs, that may be acceptable in some western countries, are hardly affordable in underdeveloped countries, and limit the access of patients to ERT.

Innovative therapeutic approaches to genetic diseases: rescuing mutated proteins with pharmacological chaperones

To circumvent some of the limitations of ERT, new therapeutic avenues have been explored in recent years. An approach that has attracted much interest as an emerging strategy for the treatment of loss-of-function genetic diseases, including LSDs, is PCT with small molecule compounds.

Missense mutations often result in the inability of protein to fold efficiently into their native conformation, that is the most energetically favourable state, thus leading to either accumulation of toxic aggregates (with a gain of function effect) or to increased degradation (with loss of metabolic or cellular functions) (Arakawa et al, 2006).

The endoplasmic reticulum (ER) has a quality control system for newly synthesized proteins, consisting of molecular chaperones that facilitate the correct protein conformation, and sensor molecules that recognize and tag incompletely folded proteins. Incorrectly folded proteins are thus retrotranslocated into the cytosol and degraded by the ER-associated degradation (ERAD) machinery (Ellgaard & Helenius, 2003).

PCT is based on the concept that ligands, such as substrates or active-site directed competitive inhibitors, may assist the folding of mutated enzymes that retain their catalytic activity, and prevent their recognition by the quality control systems (Fan & Ishii, 2007). The result of this 'pharmacological chaperone' effect is an increased intracellular pool of active enzyme, improved trafficking of the protein towards its final destination and partial restoration of the metabolic functions (Fig 2).

Besides LSDs, PCT-based therapeutic approaches have been proposed for other genetic disorders, caused both by toxicity of aggregate-prone proteins and by loss-of-function of enzymes and carriers (see Box 1)

LSDs as candidates for pharmacological chaperone therapy (PCT)

A pioneering study by Fan et al (1999) demonstrated for the first time, a chaperone effect of an imino sugar in lymphoblasts from patients with Fabry disease, caused by the deficiency of the lysosomal hydrolase α-galactosidase A (GLA) that results in vascular endothelial damage, peripheral neuropathy, cardiac, renal and eye disease. Subsequently, other studies provided the proof of principle for the use of PCT in a few other LSDs, including Gaucher disease (Yu et al, 2007), Gangliosidoses G_{M1} (Matsuda et al, 2003) and G_{M2} (Maegawa et al, 2007; Tropak et al, 2004) and Pompe disease (Okumiya et al, 2007; Parenti et al, 2007) (Table 3). Recently, Ficko-Blean et al suggested the use of chaperones also for MPS IIIB (Ficko-Blean et al, 2008).

For several reasons, LSDs can be considered excellent candidates for chaperone-mediated PCT. It is assumed that a threshold activity of approximately 10% is sufficient to prevent storage in LSDs (Schueler et al, 2004). Thus, even a minor increase in enzyme activity obtained by PCT is likely to have an impact on disease pathology and be beneficial for patients.

In addition, the use of pharmacological chaperones has the potential to overcome several ERT limitations such as its impact on the patients' quality of life and high costs. These drugs can be ingested orally and do not require invasive life-long infusions, as for ERT. One should note however, that a few adverse effects are known for some of these molecules, such as *N*-butyl-deoxynojirimycin (NB-DNJ), and we do not know much about the effect of long-term treatment. In what concerns the short term effects though, these appear to be easily managed by transient dosage reduction or diet modifications.

As far as costs go, it is reasonable to predict that the production of small molecule drugs will be less expensive than recombinant enzymes. Unfortunately, this does not seem to be the case for NB-DNJ, the commercially available compound discussed above that has potential as a pharmacological chaperone in Pompe and Gaucher disease. The treatment of a single adult patient with NB-DNJ can be estimated to be

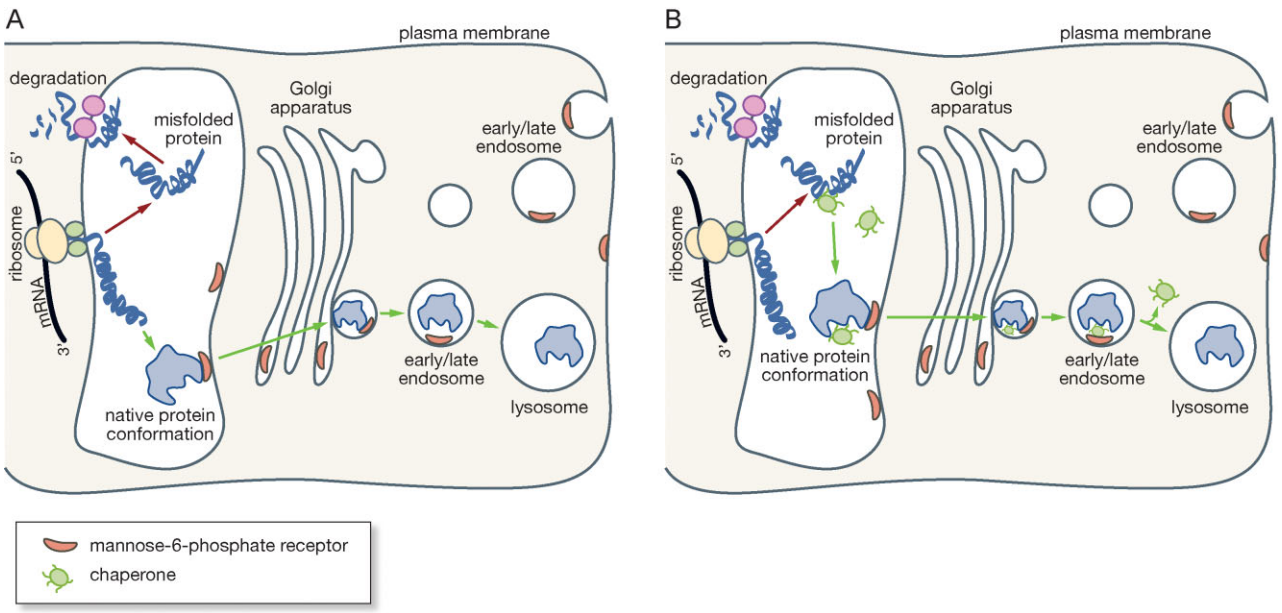

Figure 2. Effect of pharmacological chaperones on misfolded lysosomal enzymes.

A. Folding of lysosomal enzymes is assisted by endogenous molecular chaperones. While wild type enzymes are properly folded by the chaperones and correctly transported to their destination (green arrows), mutated enzymes fail to fold efficiently into their native conformation, and are retro-translocated into the cytosol and degraded by the ERAD machinery (red arrows).

B. Pharmacological chaperones favour the folding of mutated enzymes that retain their catalytic activity, and prevent their recognition by the quality control system. The complex chaperone-mutated enzyme is delivered, at least partially, to the lysosomal compartment, where the chaperone is displaced from the active site of the enzyme by the excess natural substrate.

BOX1: PCT in genetic diseases

PCT-based therapeutic approaches have been proposed both for the treatment of diseases caused by the toxic effect of aggregate-prone proteins (Kontsekova et al, 2009; Nagai & Popiel, 2008) and for a number of loss-of-function genetic disorders, such as cystic fibrosis (CF), phenylketonuria (PKU) (Pey et al, 2008), autosomal dominant retinitis pigmentosa (Bonapace et al, 2004), nephrogenic diabetes insipidus (Bernier et al, 2006; Nedvetsky et al, 2009), hyperoxaluria type I (Hopper et al, 2008).

Of these disorders, CF and PKU are particularly interesting for their prevalence and for their impact on patients' health and quality of life. Despite improved medication protocols and management, CF morbidity and mortality remains high. The ΔF508, the most prevalent mutation of the CF trans-membrane conductance regulator (CFTR) protein is potentially functional, but is misfolded and is not delivered to the plasma membrane. It has been shown that quinazoline derivatives (so called CF correctors) are able to rescue the folding and the trafficking of the ΔF508 mutant protein, restoring trans-membrane conductance (Wang et al, 2008). This strategy may allow curing the majority of CF patients, as the ΔF508 mutation accounts for 70% of disease causing alleles and is found at least in one allele in 90% of Caucasian patients.

In PKU, caused by mutations of the phenylalanine hydroxylase (PAH) gene, early detection and treatment of patients through newborn screening and restriction of phenylalanine intake have been remarkably successful in changing patients' outcome. However, diet has to be maintained for life and is expensive. Recently, Pey et al (2008) identified two potential chaperone molecules that are able to improve PAH stability and residual enzyme activity, thus opening the way to the use of PCT as a novel therapeutic approach to PKU. Nephrogenic diabetes insipidus (NDI) has also emerged as another candidate for an EET-based treatment. This disorder, characterized by a massive loss of hypo-osmotic urine, is caused by defects of aquaporin 2 (AQP2) trafficking. Thus, the development of drugs targeting AQP2 trafficking and cellular localization may have a potential for the treatment of NDI (Bernier et al, 2006; Nedvetsky et al, 2009).

Table 3. PCT in LSDs

Disease	Enzyme deficiency	Chaperone(s)	Other available therapies
Fabry disease	GLA	DGJ, galactose, 1-DGJ-lysine, galactostatin bisulphite	ERT
Gaucher disease	GBA	IFG, NB-DNJ, DNJ, NOV, 2,5-anhydro-2,5-imino-D-glucitol	ERT, SRT
G_{M1} gangliosidosis	GLB1	NOEV	—
G_{M2} gangliosidosis	HEXA	Pyrimethamine	—
Pompe disease	GAA	DNJ, NB-DNJ	ERT

NOV: *N*-octyl-beta-valienamine.

approximately 210,000 US dollars per year. It is possible that other molecules, like 1-deoxygalactonojirimycin (DGJ) used for Fabry disease therapy and second-generation compounds will be less expensive.

A major advantage of chaperones is their better cellular and tissue distribution as compared to ERT. Chaperones are small molecules and it is commonly assumed that they penetrate cell membranes and achieve therapeutic concentrations in specific cell compartments. Indeed, the data available on tissue and fluid distribution of these molecules show that they are widely distributed in tissues and organs. In rats, NB-DNJ was widely distributed in organs and in the animal carcasses (Treiber et al, 2007).

These chaperones can cross the BBB, and therefore have a potential for the treatment of diseases with CNS involvement. An important example of this potential has been reported in the mouse model of G_{M1} gangliosidosis (Suzuki et al, 2007), a severe LSD caused by β-galactosidase deficiency resulting in both visceral and neurological manifestations. In this study, a synthetic chaperone of β-galactosidase, *N*-octyl-4-epi-β-valienamine (NOEV), was rapidly and efficiently delivered to the brain and was able to increase enzyme levels in almost all areas of the brain, prevent ganglioside storage and delay neurological deterioration.

On the other hand, there is limited data on the intracellular distribution, and specifically on ER delivery, a crucial aspect for the efficacy of these drugs. To be efficient, sufficient chaperone concentrations need to be maintained in the ER lumen (Butters et al, 2005). In addition, as chaperones interact with the active site and reversibly inhibit enzyme activity, their amount in the ER is a critical factor. One must carefully consider the balance between the ER concentration required for enhancing protein folding and the concentration that inhibits the enzyme of interest or other ER-resident enzymes. Another assumption is that once in the lysosome, the chaperone is displaced from the enzyme due to the excess of natural substrate, with a higher affinity to the catalytic sites of enzymes and that the acidic environment of lysosomes favours the dissociation of the chaperone–enzyme complex. All these aspects are not completely clear and will need further investigation.

Understanding the mechanisms of chaperone–enzyme interactions

Although the rationale of PCT is clear and proof-of-concept studies for its use in LSDs have been performed, the molecular mechanisms underlying the effect of chaperones have remained speculative for years. Two alternative models have been suggested. Pharmacological chaperones may stabilize the native or native-like state of proteins or, alternatively, they may bind to proteins in non-native state and facilitate their transition to the native-like conformation (Arakawa et al, 2006).

Valuable insight was provided by recent studies on the molecular interactions of three lysosomal enzymes, GLA, GBA and GAA, with their respective chaperones. The structure of these enzymes has been solved either by crystal diffraction analysis or predicted by generating homology models (Lieberman et al, 2007, 2009; Sugawara et al, 2009; Yoshimizu et al, 2008). These enzymes share a triosephosphateisomerase (TIM) barrel $(\alpha/\beta)_8$ motif in the catalytic domain. Like the natural substrates, chaperones fit into the active site pocket and undergo hydrogen bonding with specific amino acid residues. The nature and strength of interactions between chaperones and the active site have been elucidated and a stabilizing effect of chaperones has been demonstrated (Tropak et al, 2008).

The effects of chaperone binding have been studied in detail for GBA (Lieberman et al, 2007). GBA stability and conformation were analysed in neutral and in acidic pH environments, reflective of the ER and of the lysosomal compartments, respectively, and in complex with its pharmacological chaperone isofagomine (IFG). Although the overall conformations assumed by GBA under these conditions were highly similar, small but critical differences in two loops localized at the mouth of active site were induced both by pH changes and by IFG binding. In addition, it was observed that, upon folding in the ER, GBA may assume different intermediate configurations and that IFG favours one of them. This effect of IFG on GBA conformation is important for its chaperoning function, as it appears to be favourable for enzyme stability, correct trafficking and binding of saposin C and lipids.

These studies assume clinical relevance as they give the possibility to analyse the effects of amino acid substitutions and to predict whether mutations found in patients will respond to a particular chaperone.

Specific amino acid residues have a critical role for protein conformation. In GBA, the Asn residue at position 370 is important for stabilization of the conformational change of one of the two loops at the mouth of the catalytic site. Substitution of this residue with a serine (the N370S mutation, highly prevalent in attenuated phenotypes of Gaucher disease and in Ashkenazi patients) leads to destabilization of one of the loops located in the vicinity of the catalytic site, with effects on substrate

binding. IFG binding restores proper conformation and trafficking of N370S mutated GBA (Lieberman et al, 2007).

Similarly, in Pompe disease, the location and the nature of amino acid substitution plays critical roles in determining whether a GAA variant is likely to be responsive to pharmacological chaperones (Flanagan et al, in preparation). In a screening of 76 GAA mutations, 16 of them were found to be responsive to the chaperone 1-deoxynojirimycin (DNJ). Half of the GAA residues responsive to chaperones were located in two short regions that interrupt the catalytic domain and are likely to be important determinants of substrate specificity for various glucosidases.

On the other hand, a screening of 75 different GLA missense mutations in cultured lymphoblasts from males with Fabry disease (Benjamin et al, 2009) showed that responsive amino acid substitutions were located throughout the structural domains of the enzyme and that response to chaperones was mostly associated with attenuated clinical forms of the disease.

A possible limitation of PCT in LSD emerged from extensive *in vitro* screenings of disease-causing mutations in Fabry (Benjamin et al, 2009), Gaucher (Sawkar et al, 2005) and Pompe diseases (Flanagan et al, in preparation). Based on these studies, one can estimate the fraction of LSD patients amenable to the PCT approach. Whereas in Gaucher disease, chaperone-responsive mutations are relatively frequent, such as the N370S that accounts for 34% of Gaucher alleles in non-Ashkenazi patients and 71% in Ashkenazi patients (Koprivica et al, 2000), this is not the case in the other diseases. In Pompe disease, the fraction of patients amenable to PCT would vary from 10 to 15%. In Fabry disease, most mutations are private mutations and an estimate of the rate of responsive mutations is difficult to obtain.

Towards clinical translation of PCT

After elucidating the concept of PCT in pre-clinical studies, research is now moving into clinical translation. A first patient with the cardiac variant of Fabry disease and with a relatively high GLA residual activity was treated with regular (every other day) infusions of galactose, that acted as a GLA chaperone. The patient showed improved cardiac function and histology (Frustaci et al, 2001); however, the route of administration used in this study and the frequency of infusions were not advantageous as compared to ERT.

Phase I and II clinical trials are being conducted for Fabry, Gaucher and Pompe diseases and information about these studies is available at the USA Clinical Trials website (http://www.clinicaltrials.gov). In a trial of 27 patients with Fabry disease, treated for up to 2 years with DGJ (Migalastat), the drug was safe and well tolerated. Migalastat increased the leukocyte, kidney and skin GLA activities and reduced the substrate (GL-3) levels in the urine and kidney biopsies of 24 patients. Importantly, the chaperone response of patients was comparable with *in vitro* responsiveness of *GLA* gene mutations.

Thirty patients with Gaucher disease were enrolled in a short-term trial with the chaperone AT2101. The drug was generally well tolerated and increased the GBA activity in white blood cells in 20 patients. Relevant markers of Gaucher disease remained unchanged, indicating that, at least for the 4-week duration of the study, the drug was effective in maintaining clinical stability.

Improving the treatment of patients with combination therapies

Given the limitations of all therapeutic approaches so far explored to treat LSDs, looking for new strategies to optimize therapies and to target the diverse cellular and phenotypic aspects of these diseases is becoming an important goal of current research.

In this respect, a leap forward was made, thanks to recent studies that indicated new solutions to improve the efficacy of treatments for LSDs and opened new avenues to the use of PCT. Although the concept of PCT is based on the enhancing effect of chaperones on mutated misfolded proteins, some evidence points to a possible effect of chaperones on wild-type enzymes. Chaperones induce conformational stabilization and increase thermal stability of normal recombinant enzymes (Kornhaber et al, 2008; Tropak et al, 2008).

In addition, not only mutated enzymes, but also recombinant enzymes used for ERT may be prone to mistrafficking and degradation. A study of a mouse model of Gaucher disease showed that only a fraction of the infused enzyme was recovered from animal tissues, suggesting that proteolytic degradation and/or denaturation may affect the efficacy of ERT (Xu et al, 1996). Similarly, in Pompe disease human fibroblasts and in muscle cells of a Pompe mouse model, a fraction of the rhGAA is mistargeted and is ineffective (Cardone et al, 2008; Fukuda et al, 2006).

Shen et al suggested that protection of recombinant enzymes from degradation can be achieved by chaperones. Preincubation of GBA with IFG significantly improved enzyme stability to heat, neutral pH and denaturing agents *in vitro* and resulted in increased uptake of the enzyme by cultured cells (Shen et al, 2008). Porto et al have recently provided *in vitro* and *in vivo* proof of principle that the combination of rhGAA and the chaperone NB-DNJ is more efficient in correcting the enzyme activity than rhGAA alone (Fig 3) (Porto et al, 2009). The chaperone increased the enzyme's stability *in vitro*, suggesting the possibility to obtain sustained corrective levels of the enzyme in patient's cells. Importantly, NB-DNJ also increased the delivery of the recombinant enzyme to the lysosomes. Improving the trafficking of the enzyme to lysosomes is an important therapeutic goal as (1) this organelle is the site of glycogen storage and (2) it is in the late endosomal/lysosomal compartment that GAA is processed into its 76 and 70 kDa mature and active isoforms. Improved enzyme correction in muscle, the main site of pathology in Pompe disease, was also found *in vivo* in the mouse model of Pompe disease treated with the combination of rhGAA and oral NB-DNJ (Porto et al, 2009). The synergistic effect of chaperones and ERT was not limited to Pompe disease, but was also seen in fibroblasts from a patient with Fabry disease, using the combination of rhGLA

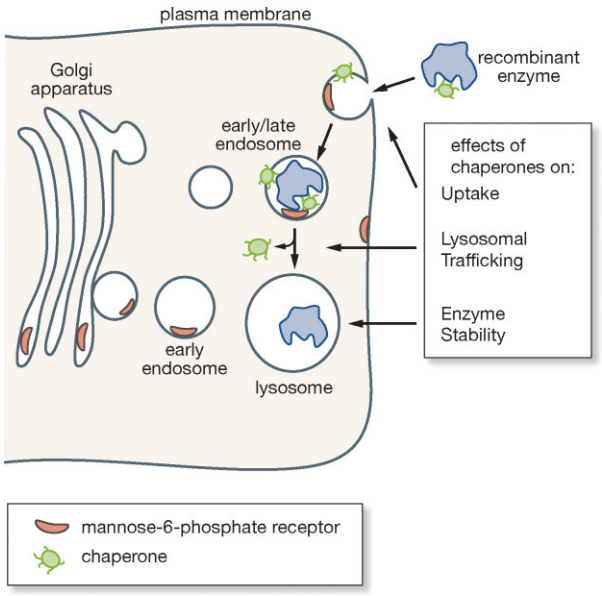

Figure 3. Synergy of ERT and chaperones. The enhancing effects of chaperones may take place at different levels.

A. By improving the uptake of the recombinant enzymes by mutant cells,

B. Favouring their intracellular trafficking to lysosomes and

C. Increasing the enzyme's stability. Improved lysosomal trafficking is an important therapeutic goal, as these organelles are the site of substrate storage and because in the late endosomal/lysosomal compartment enzymes are processed into the mature and active isoforms. Increased stability may result in sustained corrective levels of intracellular enzymes.

and the GLA chaperone DGJ (Porto et al, 2009). In contrast, NB-DNJ had no effect on rhGLA efficacy, indicating that the enhancing effect of chaperones requires specific interactions with lysosomal enzymes. These studies have important clinical implications, as they indicate a strategy to increase the efficacy of ERT. In addition, these results greatly expand the possible clinical applications of chaperones and suggest a change in the use of these molecules. A synergistic effect of these treatments may be particularly useful in patients responding poorly to therapy and in tissues in which sufficient enzyme levels are difficult to obtain. On the other hand, these data indicate new avenues of investigation. It is urgent to clarify the mechanisms underlying the effect of chaperones on wild-type enzymes and address questions as to where do chaperones and enzymes come in contact and why do they confer stability to already properly folded proteins.

The idea of combination protocols in LSDs has also been proposed for ERT and SRT. This strategy was used for Gaucher disease, to obtain therapeutic effects in tissues and organs (such as bone and cartilage) unresponsive to ERT alone, or in the brain in the neuronopathic forms of the disease (Cox-Brinkman et al, 2008).

Further advances may be derived from the improved knowledge on the cellular mechanisms regulating protein homeostasis (Powers et al, 2009). Protein homeostasis (proteostasis) is maintained by a network of pathways controlling protein synthesis, folding, trafficking, aggregation, disaggregation and degradation. These complex tasks are performed through a number of mechanisms, including those involved in the regulation of ER Ca^{2+} concentration, the unfolded protein response (UPR) and the heat-shock response (HSR). Chemical agents targeting these pathways (so called proteostasis regulators) can increase their ability to cope with the synthesis of misfolding-prone proteins. These drugs would therefore represent an additional way to improve protein folding. The concept of the use of chemical approaches to ameliorate protein folding was illustrated by a study on two LSDs; Gaucher disease, due to GBA deficiency, and Tay-Sachs disease, due to the lack of β-hexosaminidase (Mu et al, 2008b). Two proteostasis regulators (celastrol and salubrinal) were used in patient-derived fibroblasts and resulted in improved activity of the defective enzymes. Enzyme activity was further increased by co-administering a GBA chaperone, even for the L444P variant, associated with the neuronopathic Gaucher phenotypes that cannot be rescued by chaperones alone.

Manipulation of intracellular Ca^{2+} levels has also been explored as a strategy to improve protein folding. Inhibitors of Ca^{2+} channels, such as diltiazem and verapamil, by increasing ER calcium concentrations and activity of Ca^{2+}-dependent endogenous molecular chaperones, were able to partially restore lysosomal enzyme folding, trafficking and activity in three distinct LSDs: Gaucher disease, Mannosidosis and mucopolysaccharidosis IIIA (Mu et al, 2008a).

These studies are particularly attractive as the drugs used in both cases are not enzyme-specific and, in principle, a single molecule can be used for the treatment of multiple diseases. As proteostasis regulators are aimed at increasing the pool of mutant proteins available for chaperone binding, therapeutic protocol-based proteostasis regulators and PCT may act synergistically.

Conclusion and future developments

A substantial amount of novel and promising data support the potential of pharmacological chaperones as therapeutic agents in LSDs.

Future efforts should be directed towards the identification of new chaperones for additional LSDs and the identification of 'second-generation' molecules with a good safety profile and better enhancing properties and intracellular distribution. An ideal compound should have weaker inhibitory effects and higher enhancing activity (Wang et al, 2009). Novel chaperone molecules should not only be advantageous in terms of improved enhancing profile, but may also increase the rate of responsive mutations by assisting the folding of enzymes with mutations in domains other than the catalytic site. The number of chaperone molecules will likely increase in the next few years: studies with high-throughput screenings represent an efficient way of identifying new compounds for new diseases (Urban et al, 2008; Zheng et al, 2007).

Further developments in the use of chaperones are in the horizon, based on the combination with other chemical approaches to ameliorate protein folding (Mu et al, 2008b).

Bridge the gap

The Gap

Different therapeutic options have been proposed or are already approved for the treatment of an increasing number of LSDs. Among those are ERT and PCT.

ERT is presently considered the standard of care for LSDs. It has considerable success in the treatment or in ameliorating the quality of life of patients with several of these diseases. However, ERT has significant limitations in terms of tissue distribution of therapeutic enzymes, impact on patients' quality of life and high costs.

PCT, a strategy that is emerging as an alternative therapy for LSDs, appears advantageous when compared to ERT, as the chaperones are better distributed in tissues, including the brain and because therapy may be administered orally. PCT, however, also has limitations. Of note, only patients with responsive mutations will be amenable to this therapy.

The Bridge

Recent studies suggest that new solutions to improve the efficacy of treatments for LSDs may lie on the combination of distinct therapies. The combination of ERT and PCT resulted in a synergistic effect in Pompe and Fabry disease models, and may be helpful in patients responding poorly to ERT and in tissues where corrective levels of recombinant enzymes are difficult to obtain. These studies greatly expand the use of pharmacological chaperones and suggest a change in the use of these drugs.

Further developments are in the horizon, based on other chemical approaches to ameliorate protein folding. These approaches target pathways maintaining the intracellular protein homeostasis (proteostasis) and increasing the intracellular pool of mutated enzyme available for chaperone binding. Combining such approaches with chaperones will likely lead to further therapeutic improvements and more efficient correction of disease manifestations.

These approaches are aimed at modulating the degradation machinery of cells, thus increasing the intracellular pool of mutated enzyme that can be stabilized by chaperones. Combining such approaches with chaperones is feasible and likely to lead to further therapeutic improvements. The limitations related to the restricted number of patients with mutations responsive to EET may also be bypassed in the future by using combination protocols with ERT. We trust that the combination of the different therapeutic approaches discussed in this review will prove more efficient in curing or alleviating the many different pathological manifestations of LSDs.

Acknowledgements

I am grateful to Prof. Generoso Andria and Dr Nicola Brunetti-Pierri for critically reading the manuscript and for helpful discussions. The support of the Telethon Foundation and of the European Consortium for Lysosomal Diseases (EUCLYD, 7th Framework program, European Union) is acknowledged.

The author declares that he has no conflict of interest.

For more information

Clinical trial: AT2101 in Type 1 Gaucher Patients:
http://www.clinicaltrials.gov/ct2/show/NCT00813865

Clinical trial: Pharmacological Chaperones in Cells From Patients with Pompe Disease:
http://www.clinicaltrials.gov/ct2/show/NCT00515398

Clinical trial: AT1001 in Patients with Fabry Disease:
http://www.clinicaltrials.gov/ct2/show/NCT00214500

Canadian Fabry Association:
http://www.fabrycanada.com

References

Andersson U, Smith D, Jeyakumar M, Butters TD, Borja MC, Dwek RA, Platt FM (2004) Improved outcome of N-butyldeoxygalactonojirimycin-mediated substrate reduction therapy in a mouse model of Sandhoff disease. Neurobiol Dis. 16: 506-515

Arakawa T, Ejima D, Kita Y, Tsumoto K (2006) Small molecule pharmacological chaperones: From thermodynamic stabilization to pharmaceutical drugs. Biochim Biophys Acta 1764: 1677-1687

Ballabio A, Gieselmann V (2009) Lysosomal disorders: from storage to cellular damage. Biochim Biophys Acta 1793: 684-696 Epub 2008 Dec 8

Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoff CE, *et al* (1991) Replacement therapy for inherited enzyme deficiency–macrophage-targeted glucocerebrosidase for Gaucher's disease. N Engl J Med 324: 1464-1470

Benjamin ER, Flanagan JJ, Schilling A, Chang HH, Agarwal L, Katz E, Wu X, Pine C, Wustman B, Desnick RJ, *et al* (2009) The pharmacological chaperone 1-deoxygalactonojirimycin increases alpha-galactosidase A levels in Fabry patient cell lines. J Inherit Metab Dis 32: 424-440

Bernier V, Morello JP, Zarruk A, Debrand N, Salahpour A, Lonergan M, Arthus MF, Laperrière A, Brouard R, Bouvier M, *et al* (2006) Pharmacologic chaperones as a potential treatment for X-linked nephrogenic diabetes insipidus. J Am Soc Nephrol 17: 232-243

Beutler E (2006) Lysosomal storage diseases: natural history and ethical and economic aspects. Mol Genet Metab 88: 208-215

Bonapace G, Waheed A, Shah GN, Sly WS (2004) Chemical chaperones protect from effects of apoptosis-inducing mutation in carbonic anhydrase IV identified in retinitis pigmentosa 17. Proc Natl Acad Sci USA 101: 12300-12305

Brady RO (2006) Enzyme replacement for lysosomal diseases. Annu Rev Med 57: 283-296

Butters TD, Dwek RA, Platt FM (2005) Imino sugar inhibitors for treating the lysosomal glycosphingolipidoses. Glycobiology 15: 43R-52R

Cardone M, Porto C, Tarallo A, Vicinanza M, Rossi B, Polishchuk E, Donaudy F, Andria G, De Matteis MA, Parenti G (2008) Abnormal mannose-6-phosphate receptor trafficking impairs recombinant alpha-glucosidase uptake in Pompe disease fibroblasts. Pathogenetics 1: 6

Cox-Brinkman J, van Breemen MJ, van Maldegem BT, Bour L, Donker WE, Hollak CE, Wijburg FA, Aerts JM (2008) Potential efficacy of enzyme replacement and substrate reduction therapy in three siblings with Gaucher disease type III. J Inherit Metab Dis 31: 745-752

Douillard-Guilloux G, Raben N, Takikita S, Batista L, Caillaud C, Richard E (2008) Modulation of glycogen synthesis by RNA interference: towards a new therapeutic approach for glycogenosis type II. Hum Mol Genet 17: 3876-3886

Ellgaard L, Helenius A (2003) Quality control in the endoplasmic reticulum. Nat Rev Mol Cell Biol 4: 181-191

Elliot-Smith E, Speak AO, Lloyd-Evans E, Smith DA, van der Spoel AC, Jeyakumar M, Butters TD, Dwek RA, d'Azzo A, Platt FM (2008) Beneficial effects of substrate reduction therapy in a mouse model of GM1 gangliosidosis. Mol Genet Metab 94: 204-211

Fan JQ, Ishii S, Asano N, Suzuki Y (1999) Accelerated transport and maturation of lysosomal alpha-galactosidase A in Fabry lymphoblasts by an enzyme inhibitor. Nat Med 5: 112-115

Fan JQ, Ishii S (2007) Active-site-specific chaperone therapy for Fabry disease. Yin and Yang of enzyme inhibitors. FEBS J 274: 4962-4971

Ficko-Blean E, Stubbs KA, Nemirovsky O, Vocadlo DJ, Boraston AB (2008) Structural and mechanistic insight into the basis of mucopolysaccharidosis IIIB. Proc Natl Acad Sci USA 105: 6560-6565

Frustaci A, Chimenti C, Ricci R, Natale L, Russo MA, Pieroni M, Eng CM, Desnick RJ (2001) Improvement in cardiac function in the cardiac variant of Fabry's disease with galactose-infusion therapy. N Engl J Med 345: 25-32

Fukuda T, Ahearn M, Roberts A, Mattaliano RJ, Zaal K, Ralston E, Plotz PH, Raben N (2006) Autophagy and mistargeting of therapeutic enzyme in skeletal muscle in Pompe disease. Mol Ther 14: 831-839

Futerman AH, van Meer G (2004) The cell biology of lysosomal storage disorders. Nat Rev Mol Cell Biol 5: 554-565

Grubb JH, Vogler C, Levy B, Galvin N, Tan Y, Sly WS (2008) Chemically modified beta-glucuronidase crosses blood-brain barrier and clears neuronal storage in murine mucopolysaccharidosis VII. Proc Natl Acad Sci USA 105: 2616-2621

Hopper ED, Pittman AM, Fitzgerald MC, Tucker CL (2008) *In vivo* and *in vitro* examination of stability of primary hyperoxaluria-associated human alanine:glyoxylate aminotransferase. J Biol Chem 283: 30493-33502

Kontsekova E, Ivanovova N, Handzusova M, Novak M (2009) Chaperone-like antibodies in neurodegenerative tauopathies: implication for immunotherapy. Cell Mol Neurobiol [Epub ahead of print]

Koprivica V, Stone DL, Park JK, Callahan M, Frisch A, Cohen IJ, Tayebi N, Sidransky E (2000) Analysis and classification of 304 mutant alleles in patients with type 1 and type 3 Gaucher disease. Am J Hum Genet 66: 1777-1786

Kornhaber GJ, Tropak MB, Maegawa GH, Tuske SJ, Coales SJ, Mahuran DJ, Hamuro Y (2008) Isofagomine induced stabilization of glucocerebrosidase. Chembiochem 9: 2643-2649

Lieberman RL, Wustman BA, Huertas P, Powe AC, Jr, Pine CW, Khanna R, Schlossmacher MG, Ringe D, Petsko GA (2007) Structure of acid beta-glucosidase with pharmacological chaperone provides insight into Gaucher disease. Nat Chem Biol 3: 101-107

Lieberman RL, D'aquino JA, Ringe D, Petsko GA (2009) Effects of pH and iminosugar pharmacological chaperones on lysosomal glycosidase structure and stability. Biochemistry 48: 4816-4827

Maegawa GH, Tropak M, Buttner J, Stockley T, Kok F, Clarke JT, Mahuran DJ (2007) Pyrimethamine as a potential pharmacological chaperone for late-onset forms of GM2 gangliosidosis. J Biol Chem 282: 9150-9161

Matsuda J, Suzuki O, Oshima A, Yamamoto Y, Noguchi A, Takimoto K, Itoh M, Matsuzaki Y, Yasuda Y, Ogawa S, *et al* (2003) Chemical chaperone therapy for brain pathology in (GM1)-gangliosidosis. Proc Natl Acad Sci USA 100: 15912-15917

Meikle PJ, Hopwood JJ, Clague AE, Carey WF (1999) Prevalence of lysosomal storage disorders. JAMA 281: 249-254

Munoz-Rojas MV, Vieira T, Costa R, Fagondes S, John A, Jardim LB, Vedolin LM, Raymundo M, Dickson PI, Kakkis E, *et al* (2008) Intrathecal enzyme replacement therapy in a patient with mucopolysaccharidosis type I and symptomatic spinal cord compression. Am J Med Genet A 146A: 2538-2544

Mu TW, Fowler DM, Kelly JW (2008a) Partial restoration of mutant enzyme homeostasis in three distinct lysosomal storage disease cell lines by altering calcium homeostasis. PLoS Biol 6: e26

Mu TW, Ong DS, Wang YJ, Balch WE, Yates , JR 3rd, Segatori L, Kelly JW (2008b) Chemical and biological approaches synergize to ameliorate protein-folding diseases. Cell 134: 769-781

Nedvetsky PI, Tamma G, Beulshausen S, Valenti G, Rosenthal W, Klussmann E (2009) Regulation of aquaporin-2 trafficking. Handb Exp Pharmacol 190: 133-157

Nagai Y, Popiel HA (2008) Conformational changes and aggregation of expanded polyglutamine proteins as therapeutic targets of the polyglutamine diseases: exposed beta-sheet hypothesis. Curr Pharm Des 14: 3267-3279

Okumiya T, Kroos MA, Vliet LV, Takeuchi H, Van der Ploeg AT, Reuser AJ (2007) Chemical chaperones improve transport and enhance stability of mutant alpha-glucosidases in glycogen storage disease type II. Mol Genet Metab 90: 49-57

Orchard PJ, Blazar BR, Wagner J, Charnas L, Krivit W, Tolar J (2007) Hematopoietic cell therapy for metabolic disease. J Pediatr 151: 340-346

Osborn MJ, McElmurry RT, Peacock B, Tolar J, Blazar BR (2008) Targeting of the CNS in MPS-IH using a nonviral transferrin-alpha-L-iduronidase fusion gene product. Mol Ther 16: 1459-1466

Parenti G, Zuppaldi A, Pittis MG, Tuzzi MR, Annunziata I, Meroni G, Porto C, Donaudy F, Rossi B, Rossi M, *et al* (2007) Pharmacological enhancement of mutated alpha-glucosidase activity in fibroblasts from patients with Pompe disease. Mol Ther 15: 508-514

Pastores GM, Barnett NL, Kolodny EH (2005) An open-label, noncomparative study of miglustat in type I Gaucher disease: efficacy and tolerability over 24 months of treatment. Clin Ther 27: 1215-1227

Patterson MC, Vecchio D, Prady H, Abel L, Wraith JE (2007) Miglustat for treatment of Niemann-Pick C disease: a randomised controlled study. Lancet Neurol 6: 765-772

Pey AL, Ying M, Cremades N, Velazquez-Campoy A, Scherer T, Thöny B, Sancho J, Martinez A (2008) Identification of pharmacological chaperones as potential therapeutic agents to treat phenylketonuria. J Clin Invest 118: 2858-2867

Platt FM, Jeyakumar M (2008) Substrate reduction therapy. Acta Paediatr Suppl 97: 88-93

Piotrowska E, Jakóbkiewicz-Banecka J, Barańska S, Tylki-Szymańska A, Czartoryska B, Wegrzyn A, Wegrzyn G (2006) Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. Eur J Hum Genet 14: 846-852

Porto C, Cardone M, Fontana F, Rossi B, Tuzzi MR, Tarallo A, Barone MV, Andria G, Parenti G (2009) The pharmacological chaperone N-butyldeoxynojirimycin enhances enzyme replacement therapy in Pompe disease fibroblasts. Mol Ther 17: 964-971

Powers ET, Morimoto RI, Dillin A, Kelly JW, Balch WE (2009) Biological and chemical approaches to diseases of proteostasis deficiency. Annu Rev Biochem 78: 959-991

Roberts AL, Rees MH, Klebe S, Fletcher JM, Byers S (2007) Improvement in behaviour after substrate deprivation therapy with rhodamine B in a mouse model of MPS IIIA. Mol Genet Metab 92: 115-121

Sands MS, Davidson BL (2006) Gene therapy for lysosomal storage diseases. Mol Ther 13: 839-849

Suzuki Y, Ichinomiya S, Kurosawa M, Ohkubo M, Watanabe H, Iwasaki H, Matsuda J, Noguchi Y, Takimoto K, Itoh M, *et al* (2007) Chemical chaperone therapy: clinical effect in murine GM1-gangliosidosis. Ann Neurol 62: 671-675

Sawkar AR, Adamski-Werner SL, Cheng WC, Wong CH, Beutler E, Zimmer KP, Kelly JW (2005) Gaucher disease-associated glucocerebrosidases show mutation-dependent chemical chaperoning profiles. Chem Biol 12: 1235-1244

Schueler UH, Kolter T, Kaneski CR, Zirzow GC, Sandhoff K, Brady RO (2004) Correlation between enzyme activity and substrate storage in a cell culture model system for Gaucher disease. J Inherit Metab Dis 27: 649-658

Shen JS, Edwards NJ, Hong YB, Murray GJ (2008) Isofagomine increases lysosomal delivery of exogenous glucocerebrosidase. Biochem Biophys Res Commun 369: 1071-1075

Sugawara K, Tajima Y, Kawashima I, Tsukimura T, Saito S, Ohno K, Iwamoto K, Kobayashi T, Itoh K, Sakuraba H (2009) Molecular interaction of imino sugars with human alpha-galactosidase: insight into the mechanism of complex formation and pharmacological chaperone action in Fabry disease. Mol Genet Metab 96: 233-238

Treiber A, Morand O, Clozel M (2007) The pharmacokinetics and tissue distribution of the glucosylceramide synthase inhibitor miglustat in the rat. Xenobiotica 37: 298-314

Tropak MB, Reid SP, Guiral M, Withers SG, Mahuran D (2004) Pharmacological enhancement of beta-hexosaminidase activity in fibroblasts from adult Tay-Sachs and Sandhoff patients. J Biol Chem 279: 13478-13487

Tropak MB, Kornhaber GJ, Rigat BA, Maegawa GH, Buttner JD, Blanchard JE, Murphy C, Tuske SJ, Coales SJ, Hamuro Y, *et al* (2008) Identification of pharmacological chaperones for Gaucher disease and characterization of their effects on beta-glucocerebrosidase by hydrogen/deuterium exchange mass spectrometry. Chembiochem 9: 2650-2662

Urban DJ, Zheng W, Goker-Alpan O, Jadhav A, Lamarca ME, Inglese J, SidranskyE, Austin CP (2008) Optimization and validation of two miniaturized glucocerebrosidase enzyme assays for high throughput screening. Comb Chem High Throughput Screen 11: 817-824

van der Ploeg AT, Reuser AJ (2008) Pompe's disease. Lancet 372: 1342-1353

Wang GN, Reinkensmeier G, Zhang SW, Zhou J, Zhang LR, Zhang LH, Butters TD, Ye XS (2009) Rational design and synthesis of highly potent pharmacological chaperones for treatment of N370S mutant Gaucher disease. J Med Chem 52: 3146-3149

Wang X, Koulov AV, Kellner WA, Riordan JR, Balch WE (2008) Chemical and biological folding contribute to temperature-sensitive DeltaF508 CFTR trafficking. Traffic 9: 1878-1893

Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, Dyke JP, Ballon D, Heier L, Greenwald BM, *et al* (2008) Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. Hum Gene Ther 19: 463-474

Wraith JE (2006) Limitations of enzyme replacement therapy: current and future. J Inher Metab Dis 29: 442-447

Yoshimizu M, Tajima Y, Matsuzawa F, Aikawa S, Iwamoto K, Kobayashi T, Edmunds T, Fujishima K, Tsuji D, Itoh K, *et al* (2008) Binding parameters and thermodynamics of the interaction of imino sugars with a recombinant human acid alpha-glucosidase(alglucosidase alfa): insight into the complex formation mechanism. Clin Chim Acta 391: 68-73

Yu Z, Sawkar AR, Kelly JW (2007) Pharmacologic chaperoning as a strategy to treat Gaucher disease. FEBS J 274: 4944-5017

Xu YH, Ponce E, Sun Y, Leonova T, Bove K, Witte D, Grabowski GA (1996) Turnover and distribution of intravenously administered mannose-terminated human acid beta-glucosidase in murine and human tissues. Pediatr Res 39: 313-322

Zheng W, Padia J, Urban DJ, Jadhav A, Goker-Alpan O, Simeonov A, Goldin E, Auld D, LaMarca ME, Inglese J, *et al* (2007) Three classes of glucocerebrosidase inhibitors identified by quantitative high-throughput screening are chaperone leads for Gaucher disease. Proc Natl Acad Sci USA 104: 13192-13197

Zhu Y, Jiang JL, Gumlaw NK, Zhang J, Bercury SD, Ziegler RJ, Lee K, Kudo M, Canfield WM, Edmunds T, *et al* (2009) Glycoengineered acid alpha-glucosidase with improved efficacy at correcting the metabolic aberrations and motor function deficits in a mouse model of Pompe disease. Mol Ther 17: 954-963

