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REVIEW

Enzyme replacement therapy and beyond—in memoriam Roscoe O. Brady, M.D. (1923–2016)

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Abstract Lysosomal storage disorders are strong candidates for the development of specific innovative therapies. The discovery of enzyme deficiencies is an important milestone in understanding the underlying cause of disease. Being able to replace the first missing enzyme in a lysosomal storage required three decades of dedicated research. Successful drug development for lysosomal storage disorders was fostered by the U.S. Orphan Drug Act. Various optimization strategies have the potential to overcome the current limitations of enzyme replacement therapies. In addition, substrate reduction therapies are an alternative approach to treat lysosomal storage disorders, chemical chaperones enhance residual enzyme activity, and small molecules can facilitate substrate transport through subcellular compartments. Bone-marrow derived multipotent stem cells and gene therapies have received FDA orphan drug designation status. The science of small clinical trials played an essential role: non-neurological endpoints, biomarker, and regulatory alignment are key factors in successful drug development for lysosomal storage disorders. Being able to treat brain disease is the next frontier. This review is dedicated to the memory of Roscoe O. Brady, an early pioneer in the research of lysosomal storage diseases.

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Lysosomal storage disorders as candidates for the development of specific innovative therapies

Eukaryotic cells are surrounded by a plasma membrane that contains glycosphingolipids. The membrane is constantly remodeled requiring continous degradation of macromolecules and smaller substances. The final degradation products, such as sphingosine, fatty acids, monosaccharides, sialic acids, or sulfate, leave the lysosome via diffusion or transport systems and are subsequently recycled for synthesis of macromolecules or further degraded in order to provide energy. If any of the exohydrolases or an activator protein are deficient, non-degradable substrates will accumulate in the cells and cause a lysosomal storage disorder, such as sphingolipidoses, mucopolysaccharidoses (MPS), glycoprotein, or glycogen storage disease (Sandhoff and Kolter 2003; Sabatini and Adesnik 2005); Kolter and Sandhoff 2005). With an increasing understanding of molecular concepts and mechanisms of lysosomal storage diseases, a variety of therapeutical or potential therapeutical options received consideration: replacement of the missing enzyme (enzyme replacement therapy), reduction of substrate formation (substrate reduction therapy), stabilization of misfolded protein (pharmacological chaperone therapy), replacement of defect cells (stem cell transplantation), replacement of the deficient gene (gene therapy), and correction of gene transcription (stop codon read through).

The pathway toward the understanding of the underlying cause of lysosomal storage disorders led to the discovery of enzyme deficiencies

Clinical case reports of conditions that were later understood to be lysosomal storage disorders were initially published in the last quarter of the nineteenth century, such as Tay-Sachs-

disease (Tay 1881), Gaucher disease (Gaucher 1882), and Fabry disease (Anderson 1898; Fabry 1898). In 1934, the identification of accumulated glucocerebroside as the underlying mechanism of Gaucher disease by Aghion set an important milestone for identification of potential therapeutic targets and subsequent drug development (Aghion 1934). In the same year, Klenk found that sphingomyelin accumulated in patients with Niemann–Pick disease (Klenk 1934). In 1962, Svennerholm reported ganglioside G_{M2} accumulated in Tay–Sachs disease (Svennerholm 1962), and in 1963 ceramidetrihexoside was identified to accumulate in Fabry disease (Sweeley and Klionsky 1963). The concept of a lysosomal storage disorder was proposed after the discovery that the deficiency of the enzyme acid maltase causes Pompe disease (Baudhuin et al 1964). Further underlying enzyme deficiencies were discovered in the 1960s: glucocerebrosidasedeficiency in Gaucher disease (Fig. 1) (Brady et al 1965), sphingomyelinase deficiency in Niemann-Pick disease (Brady et al 1966), ceramidetrihexosidase deficiency in Fabry disease (Brady et al 1967), and the biochemical evidence of inefficient enzymatic degradation of mucopolysaccharides in MPS I and II (Fratantoni et al 1968a).

The principle of enzyme replacement therapy is based upon the observation of an "experiment of nature": in lysosomal storage disorders, such as Fabry disease, residual enzyme activity protects the patient to a certain degree from the full blown, severe classical disease phenotype. For instance, in Fabry disease, patients with residual enzyme activity exhibit a later onset of renal involvement, a decreased prevalence of neuropathic pain, a lower incidence of cornea verticillata, and a lower incidence of hearing loss compared with patients with complete enzyme deficiency (Altarescu et al 2001b; Branton et al 2002; Ries et al 2005, 2006a, b; 2007a, b2007a, b). Conzelmann and Sandhoff proposed the threshold theory of residual enzyme activity to explain the correlation between severity of the disease and residual enzyme activity. While the mutated catabolic enzyme may have residual catalytic ability, mutation specific altered kinetic properties, such as a different K_m or V_{max} may be present. Only if the velocity of substrate load into the lysosome is larger than the velocity of degradation (V_{max}) by the corresponding enzyme, glycosphingolipids will be stored. Milder forms of sphingolipidoses dispose sufficient residual enzyme activity facilitating very slow accumulation of degradation products (Conzelmann and Sandhoff 1983). This model could be verified *in vitro* in lysosomal storage disorders such as G_{M2}-gangliosidosis, metachromatic leukodystrophy (Leinekugel et al 1992), and Gaucher disease (Schueler et al 2004). Therefore, enzyme replacement therapy aims at substituting the missing enzyme to a level at least as high as the minimally effective level of residual enzyme activity and ideally be able to convert a classical clinical phenotype at least into an attenuated one.

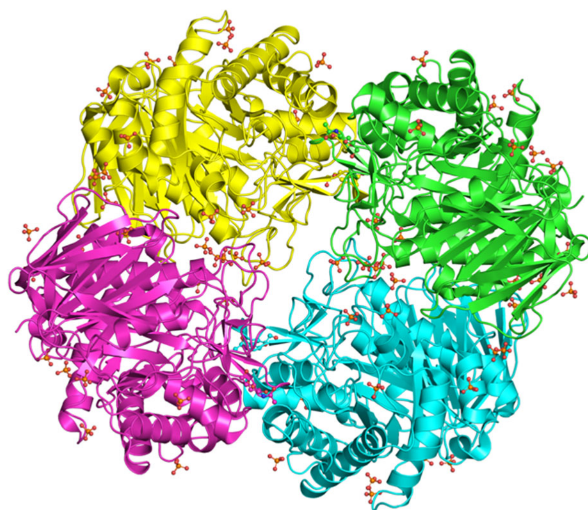

Fig. 1 Crystal structure of glucocerebrosidase (PDBe 2017)

Replacing a missing enzyme: three decades of dedicated research

Based on the observation that there is a lysosomal accumulation of degradation material caused by enzyme deficiency, the strategy to replace the missing enzyme was proposed by Christian de Duve and Roscoe Brady in the late 1960s:

> "In our pathogenic speculations and in our therapeutic attempts, it may be well to keep in mind that any substance which is taken up intracellularly in an endocytic process is likely to end up within lysosomes. This obviously opens up many possibilities for interaction, including replacement therapy" (De Duve 1964)
> "Since there is good evidence for a specific enzyme deficiency in at least three of the sphingolipidoses a potential approach might be the replacing or supplementing of these deficiencies by exogenous administration of the respective enzyme" (Brady 1966).

The translation of these visionary ideas into clinical practice would require sustained commitment for almost three decades: In 1991, the first enzyme replacement therapy, i.e., imiglucerase for Gaucher disease, received orphan drug approval by the Food and Drug Administration (FDA) (Mechler et al 2015). Mutual cross correction of the respective deficient enzyme by simultaneous cell-culture of fibroblasts from patients with MPS I and MPS II, demonstrated by the group of Elizabeth Neufeld in 1968, constitutes an early preclinical proof of concept (Fratantoni et al 1968b). The production of the therapeutic enzyme—a biological—and long-term supply for patients in

sufficient quantities was an early concern for the feasibility of this approach. Pentchev et al suggested the isolation of glucocerebrosidase from human tissue and proposed placentas as a constantly available source of material for clinical trials and long term treatment (Pentchev et al 1973; Furbish et al 1977). The research for enzyme replacement therapy at the National Institutes of Health moved forward in parallel for Gaucher disease and Fabry disease. An important milestone was the demonstration of a pharmacodynamic proof of concept: Ceramidetrihexosidase isolated from human placenta was able to induce a rapid reduction of ceramidetrihexoside in two male patients with Fabry disease (Brady et al 1973). Likewise, the administration of purified glucocerebrosidase in patients having Gaucher disease decreased glucocerebroside concentrations in the liver and that associated with red blood cells in the circulation of two patients with Gaucher disease (Brady et al 1974). In order to improve the therapeutic response, exogenous glucocerebrosidase was biochemically modified and targeted to mannose lectin on macrophages (Brady et al 1994). The next step was to demonstrate a clinical response to therapy. In one patient with type 1 Gaucher disease, hemoglobin and platelets increased and bone manifestations improved with repeated infusions of purified glucocerebrosidase (Barton et al 1990). Subsequently, a sustained therapeutic response to enzyme replacement therapy, i.e., an increase of hemoglobin and platelets, decrease in serum acid phosphatase, decrease of spleen size, and improved skeleton, was shown in a larger group of patients ($N = 12$) with Gaucher disease. These observations eventually led to the FDA approval of imiglucerase for the treatment of Gaucher disease, which was the first FDA approved orphan drug for a lysosomal storage disorder (Barton et al 1991; Mechler et al 2015). In order to further minimize infection risk and to become independent from external tissue supply, the manufacturing method was later changed from purified placenta derived enzyme to a recombinant source based on Chinese hamster ovary (CHO) cells (Grabowski et al 1995). The development of enzyme replacement therapy with agalsidase alfa was done in a similar way a decade later. First, the reduction of disease inherent storage material, i.e., globotriaosylceramide in liver tissue and shed renal tubular epithelial cells was demonstrated (Schiffmann et al 2000). Clinical efficacy and safety was first tested in a randomized clinical trial in adult patients (Schiffmann et al 2001) and subsequently investigated in children (Ries et al 2006a, b; 2007a, 2007a, b).

Successful drug development for lysosomal storage disorders was fostered by the U.S. Orphan Drug Act

The US Orphan Drug Act of 1983 was instrumental in translating biochemical and genetic discoveries into innovative therapies for patients with lysosomal storage disorders. The Orphan Drug Act intends to stimulate the investment into the development of medicines for rare diseases. Various incentives are proposed such as seven years' marketing exclusivity, tax credit for 50% of clinical trial costs, protocol assistance, FDA fee waiver, and orphan grants programs (Haffner et al 2008). By 18 January 2017, a total of 590 orphan indications were approved by the FDA (FDA) including therapies for lysosomal storage disorders (Table 1).

The four key factors that played an essential role in successful orphan drug development or orphan drug designations were prevalence of disease, endpoints in clinical trials, regulatory precedent (a drug approval in the same or a clinically very similar disease such as the MPS group), and finally the pharmacological technology platform (Mechler et al 2015). The first orphan drug designation for a compound intended to treat a lysosomal storage disorder occurred in 1985 for alglucerase which was approved by the FDA in 1991 for the treatment of Gaucher disease and became the first approved orphan drug for a lysosomal storage disorder. Successful development seeded further innovation: in the three decades between 1983 and 2013, 14 drugs for seven conditions received FDA approval (Fig. 2). By today (January 2017), there are ten lysosomal conditions with FDA and/or EMA drug approvals, i.e., Fabry disease, cystinosis, Gaucher disease, lysosomal acid lipase deficiency, Niemann-Pick disease type C, Pompe disease, MPS I, MPS II, MPS IVA, and MPS VI. Most of these FDA approved drugs were enzyme replacement therapies indicating that, until today, enzyme replacement therapy was the most successful technology. Substrate reduction therapies, and small molecules facilitating lysosomal substrate transportation were developed, too (Fig. 3). Of interest, Fabry disease, Gaucher disease and cystinosis had multiple drug approvals by the FDA and/or EMA. Successful FDA approval was significantly associated with higher disease prevalence and clinical development programs that did not require a primary neurological endpoints. As such, drug development in lysosomal storage disorders focused on more common diseases and primarily neurological lysosomal storage disorders were neglected. Small clinical trials with somatic or biomarker endpoints were successful study designs leading to FDA drug approval.

Orphan drug status was designated for enzymes, modified enzymes, fusion proteins, chemical chaperones, small molecules leading to substrate reduction, or facilitating subcellular substrate transport, stem cells as well as gene therapies (Fig. 4) (Mechler et al 2015). Therapeutic enzymes are manufactured in a variety of cell types. FDA approved enzyme replacement therapies were extracted from human placenta (Barton et al 1991), or derived from human (Muenzer et al 2007), animal (Chinese hamster ovaries), (Grabowski et al 1995; Eng et al 2001; Harmatz et al 2004; Wraith et al 2004; Kishnani et al 2007), or plant cells (carrot) production systems (Zimran et al 2011). Orphan drug status was also assigned for enzymes made in hen oviduct cells and insect cells (Chen et al 2000; Leavitt et al 2013). Although these different systems deliver enzymes

Table 1 Compounds for treatment of lysosomal storage disorders

Disease	Compound	Therapeutic class	Endpoints in pivotal studies	Pivotal trial design	Ref.	Administration and dosage	Cochrane reviews
Cystinosis	Cysteamine bitartrate IR	Small molecule	Serum creatinine, calculated creatinine clearance, growth (height)	OLT, $N = 94$, mean age 3.8 years	(FDA 2007)	Oral. Maintenance [...] maintenance dose: for children up to age 12 years: 1.30 grams/m^2/day of the free base, given in four divided doses. Patients over age 12 and over 110 pounds weight should receive 2.0 grams/day, divided four times daily.	N/A
Cystinosis	Cysteamine ophthalmic solution	Small molecule	Corneal Cystine Crystal Score	OLT, $N = 283$ (three studies)	(FDA 2012a)	Eye drops one drop in each eye, every waking hour.	N/A
Cystinosis	Cysteamine bitartrate DR	Small molecule	White blood cell cystine	RCT, $N = 43$, mean age 12 years (range 6 – 26 years)	(FDA 2013)	Oral Maintenance dose: 1.3 gram/m^2 per day, divided into two equal doses given every 12 hours.	N/A
Fabry disease	Agalsidase beta	Enzyme	Reduction of GL-3 inclusions in capillary endothelium of kidney, heart and skin	RCT, duration 20 weeks, $N = 58$, mean age 30.2 years (range 16–61 years)	(FDA 2003a)	IV 1 mg/kg every 2 weeks	(El Dib, Nascimento et al 2013)
Fabry disease	Agalsidase alfa*	Enzyme	Pain levels, renal function, fraction of normal glomeruli and fraction of glomeruli with mesangial widening, left ventricular hypertrophy, Gb_3-concentrations in plasma, urine, and cardiac biopsies	RCT, duration 6 months, $N = 26$ and $N = 15$, adult males	(EMA 2006)	IV 0,2 mg/kg every 2 weeks	(El Dib, Nascimento et al 2013)
Fabry disease	Migalastat*	Small molecule	Renal function, left ventricular mass index, plasma lyso-Gb_3, kidney interstitial capillary GL-3 inclusions, GL-3 levels were observed in multiple renal cell types, composite clinical outcome (renal, cardiac, and cerebro-vascular events, or death), Gastrointestinal Symptoms Rating Scale	OLT with active comparator (ERT), duration 18 months, $N = 52$, adult male and females with amenable mutations RCT, duration 18 months, $N = 50$ males and females above 16 years with amenable mutations	(EMA 2016)	Oral 123 mg QOD	N/A
Gaucher Disease	Miglustat	Small molecule	Liver and spleen volume change, hemoglobin concentration, platelet Count	OLT, duration 52 weeks, $N = 28$, mean age 44 years (range 22–69 years)	(FDA 2003b)	Oral 100 mg TID	(Shemesh, Deroma et al 2015)
Gaucher disease	Imiglucerase	Enzyme			(FDA 2005a)	IV	

Table 1 (continued)

Disease	Compound	Therapeutic class	Endpoints in pivotal studies	Pivotal trial design	Ref.	Administration and dosage	Cochrane reviews
			Anemia and thrombocytopenia, liver and spleen volume change, decreased cachexia	RCT, duration 26 weeks, N = 30, mean age 32.7 years (range 12 – 69 years)		15–60 U/kg every 2 weeks	(Shemesh, Deroma et al 2015)
Gaucher disease	Taliglucerase alfa[¶]	Enzyme	Hemoglobin concentration, platelet count, liver and spleen volume change	RCT, duration 36 weeks, N = 32, mean age 36.2 years (range 19 – 74 years)	(FDA 2012b)	IV 60 Units/kg every other week ranges from 11 Units/kg to 73 Units/kg every other week	(Shemesh, Deroma et al 2015)
Gaucher disease	Velaglucerase alfa	Enzyme	Hemoglobin concentration, platelet count, liver and spleen volume change	RCT, N = 25, duration 52 weeks, median age 25 years, (range 4–62 years)	(FDA 2010a)	IV 15–60 Units/kg every other week	(Shemesh, Deroma et al 2015)
Gaucher disease	Eligustat	Small molecule	Spleen volume, hemoglobin level, liver volume, platelet count	RCT, N = 40, duration 9 months, 16 years and older	(FDA 2014a)	Oral 84 mg BID (intermediate and extensive CYP2D6 metabolizier) 84 mg QD (poor CYP2D6 metabolizer)	(Shemesh, Deroma et al 2015)
Gaucher disease type I	Alglucerase	Enzyme	Liver and spleen volume change, Hematologic deficiencies, improved mineralization of bone, cachexia and wasting	OLT, duration 36 – 52 weeks, N = 13, mean age 20.3 years (range 7–42 years)	(FDA 2006a)	IV 2.5 units/kg 3 times a week up to 60 units/kg as frequently as once a week or as infrequently as every 4 wk.	(Shemesh, Deroma et al 2015)
Lysosomal Acid Lipase deficiency (Wolman disease/CESD)	Sebelipase alfa	Enzyme	Survival past 12 months of age, ALT, LDL-cholesterol, non-HDL-cholesterol, AST, triglycerides, HDL-cholesterol, decrease in liver fat content, hepatic steatosis	OLT, N = 9, age 1 to 6 months RCT, N = 66, age 4–58 years, duration 20 weeks	(FDA 2015)	IV 1 mg/kg every other week – 1–3 mg/kg weekly	N/A
MPS I	Laronidase	Enzyme	Forced vital capacity (% of predicted), 6 min walk distance	RCT, duration 26 weeks, N = 45, mean age 15.5 years (range 6 – 43 years)	(FDA 2003c)	IV 100 U/kg weekly	(Jameson, Jones et al 2013)
MPS II	Idursulfase	Enzyme	Forced vital capacity (% of predicted), 6 min walk distance	RCT, duration 53 weeks, N = 96, mean age 14.2 years (range 5–31 years)	(FDA 2006b)	IV 0.5 mg/kg weekly	(da Silva, Strufaldi et al 2014)
MPS IVA	Elosulfase alfa	Enzyme	6 min walk distance	RCT, N = 176, duration 24 weeks age 5 – 57 years	(FDA 2014b)	IV 2 mg/kg weekly	N/A
MPS VI	Galsulfase	Enzyme	12 min walk distance, 3 min stair climb test (stairs/min)	RCT, duration 24 weeks, N = 39, (age range 5–29 years)	(FDA 2005b)	IV 1 mg/kg weekly	N/A
Niemann-Pick disease type C	Miglustat*	Small molecule	Horizontal saccadic eye movement	OLT, duration 12 months, N = 29, age 12 years and older, in addition: 12 patients under 12 years of age	(EMA 2009)	Oral Children >12 years and adults 200 mg TID Children below 12 years: adjust to body surface area	N/A

Table 1 (continued)

Disease	Compound	Therapeutic class	Endpoints in pivotal studies	Pivotal trial design	Ref.	Administration and dosage	Cochrane reviews
Pompe disease	Alglucosidase alfa	Enzyme (bioreactor size: 160 L)	Number of patients who died or needed invasive ventilator support	OLT, duration 52–106 weeks, $N = 18$, age range 1 month to 3.5 years	(FDA 2006c)	IV 20 mg/kg every other week	N/A
Pompe disease	Alglucosidase alfa	Enzyme (bioreactor size: 4000 L)	Forced vital capacity (% of predicted), 6 min walking distance	RCT, duration 78 weeks, $N = 90$, mean age 44.4 years (range 10–70 years)	(FDA 2010b)	IV 20 mg/kg every other week	N/A

* - not approved by the FDA. ¶ - not approved by the EMA

RCT – randomized controlled trial, OLT – open label trial, IR – immediate release, DR – delayed-release, GL-3 / Gb_3 – globotriaosylceramide, QD – once a day, BID – twice a day, TID – three times a day, IV intravenous, CESD - Cholesteryl ester storage disease , ALT - alanine transaminase, AST - Aspartate transaminase, HDL – high density lipoprotein, LDL – low density lipoprotein, N/A – not available

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FDA. (2003b). "Drug Approval Package Zavesca (Miglustat) " Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2003/21-348_Zavesca.cfm.

FDA. (2003c). "Laronidase Product Approval Information - Licensing Action " Retrieved 20 Dec 2013, from http://www.fda.gov/Drugs/DevelopmentApprovalProcess/HowDrugsareDevelopedandApproved/ApprovalApplications/TherapeuticBiologicApplications/ucm080438.htm.

FDA. (2005a). "CEREZYME, NDA no. 020367, label " Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/label/2005/20367s066lbl.pdf.

FDA. (2005b). "Drug Approval Package: Naglazyme (Galsulfase) NDA #125117 " Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2005/125117s000_NaglazyneTOC.cfm.

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FDA. (2010b). "Drug Approval Package Lumizyme (alglucosidase alfa) ", Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2010/125291s000TOC.cfm.

FDA. (2012a). "Drug Approval Package Cystaran (cysteamine ophthalmic solution) 0.44%, NDA # 200740 " Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/200740_cystaran_toc.cfm.

FDA. (2012b). "Drug Approval Package Elelyso (Taliglucerase alfa) ", Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/nda/2012/022458Orig1s000TOC.cfm.

with different biochemical and pharmacological properties which can be tested and compared in cellular and animal models, the true clinical significance of these differences in humans remains to be investigated through appropriately designed and sufficiently powered studies or meta-analyses in human. Such a comparison can be difficult and has recently been made in children and adolescents with diabetes type I, not without eliciting a considerable debate among stake holders involved. Specifically, there were no significant differences for patient-relevant outcomes between various rapidly acting insulin analogues and human insulin (IQWiG 2005).

Optimization strategies have the potential to overcome the current limitations of enzyme replacement therapies

The effect of enzyme replacement therapy is mainly compromised by late initiation of treatment, immune reactions against the therapeutic protein and incomplete bioavailability in certain tissues, such as skeletal muscle, bone, and especially brain (Altarescu et al 2001a; Ries et al 2006a, b; Strothotte et al 2010; Ohashi 2012). Dose and frequency of enzyme administration are further important questions (Schiffmann et al 2007, 2015).

Various strategies have been employed to address the issue of the blood brain barrier. High dose intravenous enzyme replacement therapy has been proposed, however, significant effects on neurological outcomes could not be detected in a clinical study in patients with Gaucher disease type 3 (Altarescu et al 2001a; Goker-Alpan et al 2008). More recently, Ou et al reported the feasibility of this approach in MPS I mice and Blanz et al in alpha-mannosidosis mice (Blanz et al 2008; Ou et al 2014). Given the clinical experience in Gaucher disease, further studies in humans will be of particular interest. To overcome the blood brain barrier in an experimental setting, one patient with Gaucher disease type 2 was infused recombinant glucocerebrosidase directly into the brainstem by convection-enhanced delivery (CED) without signs of toxicity (Lonser et al 2007). However, long-term feasibility, safety, and efficacy of this approach require further studies. For intrathecal enzyme administration in MPS II and MPS IIIA, an orphan drug designation was granted. Iduronate-2-sulfatase was successfully administered intrathecally by intracerebroventricular and lumbar routes in healthy dogs and nonhuman primates, as well as in the MPS II mouse model (Calias et al 2012). Monthly intrathecal enzyme administration was studied in cynomolgus monkeys in a six month animal toxicology study (Felice et al 2011). A human clinical trial was conducted between 2009 and 2012 in patients with MPS II ((Muenzer et al 2016). Another clinical trial for a compound with orphan drug designation investigated intrathecal enzyme administration into the cerebrospinal fluid in patients with MPS IIIA through an intrathecal drug delivery

FDA. (2013). "PROCYSBI, NDA no. 203389, label " Retrieved 20 Dec 2013, from http://www.accessdata.fda.gov/drugsatfda_docs/label/2013/203389s000lbl.pdf.
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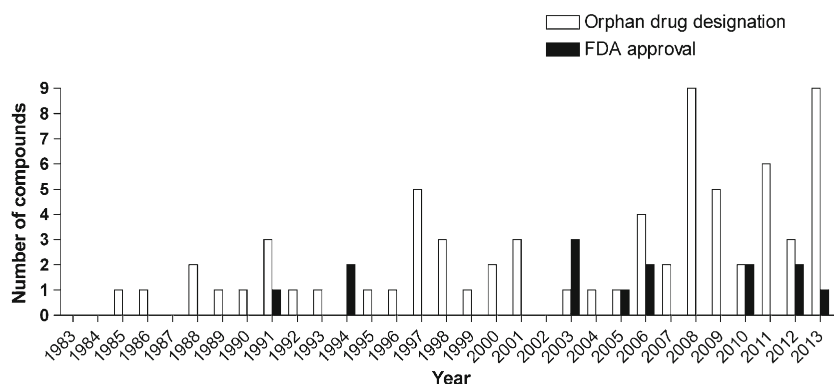

Fig. 2 Number of orphan drug designations (open bars) and FDA approvals (full bars) for compounds intended to treat lysosomal storage disorders by year (Mechler et al 2015).

device between 2010 and 2012 (Jones et al 2016). Sohn et al proposed continuous intrathecal enzyme infusions with iduronate-2-sulfatase which was tested in the MPS II mouse model instead of administering enzyme at long and therefore non-physiological time intervals (Sohn et al 2013).

Modification of therapeutic enzymes is a strategy to improve targeting properties of approved and designated orphan compounds. Human placental glucocerebrosidase (Gaucher disease) was deglycosylated to yield a macrophage-targeted mannose-terminated preparation allowing an uptake mediated by mannose receptors (Barton et al 1990). The addition of polyethylene glycol to glucocerebrosidase facilitating an increased plasma half-life was proposed and tested in a clinical trial (clinicaltrials.gov identifier NCT00001410). A PEGylated version of alpha-galactosidase A (Fabry disease) is currently under investigation in human (clinicaltrials.gov identifier NCT01678898).

Targeting the cation-independent mannose-6-phosphate receptor, recombinant human alpha glucosidase (Pompe disease) was conjugated with bis-mannose-6-phosphate bearing synthetic glycans in order to improve muscular uptake of the enzyme (Zhou et al 2011).

Orphan drug designations were granted for fusion proteins. In lyosomal storage disorders fusion proteins are enzymes coupled with another protein or part of a protein with high affinity for a certain receptor which enhances the ability for the large molecule to cross barriers between compartments. As such, recombinant human α-N-acetyl-glucosaminidase (MPS IIIB) was modified by fusing the enzyme to the receptor binding motif of insulin-like growth factor-II (IGF-II). In order to enhance the cellular and lysosomal uptake, this fusion protein is targeted at the cation-independent mannose-6-phosphate receptor, which is also the receptor for IGF-II at a different binding site (Kan et al 2014). Human acid α-glucosidase (Pompe disease) was fused to a portion of IGF-II targeting the cation-independent mannose-6-phosphate receptor in order to increase cellular uptake (Maga et al 2013). Human alpha-L-iduronidase (MPS I) and iduronate-2-sulfatase (MPS II), were each coupled with the heavy chain of a chimeric monoclonal antibody to the human insulin receptor. These fusion proteins were designed to cross the blood–brain-barrier facilitated by the endogenous insulin receptor (Boado et al 2008) and are under investigation in clinical trials in MPS I and MPS II (clinicaltrials.gov identifier NCT02371226 and NCT02262338)

Substrate reduction therapies are an alternative approach to treat lysosomal storage disorders

Substrate reduction therapies are based on small molecules that reduce the formation of substrate for a compromised enzyme by inhibiting the respective synthase of the particular non-degradable substrate. The first FDA approved substrate inhibitor was miglustat (Fig. 5) for Gaucher disease (Cox et al 2000). This compound inhibits the ceramide-specific glucosyltransferase which initiates the glycosphingolipid

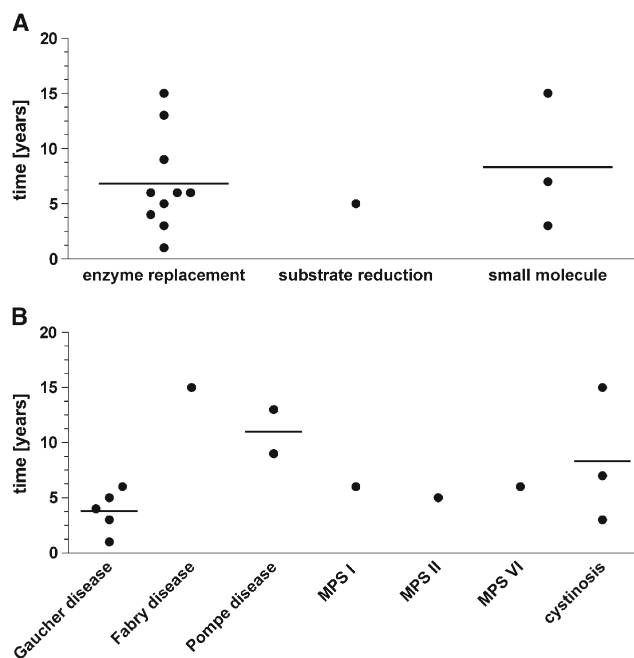

Fig. 3 Time to approval of compounds intended to treat lysosomal storage disorders by A) technology platform and B) disease. Lines indicate means (Mechler et al 2015).

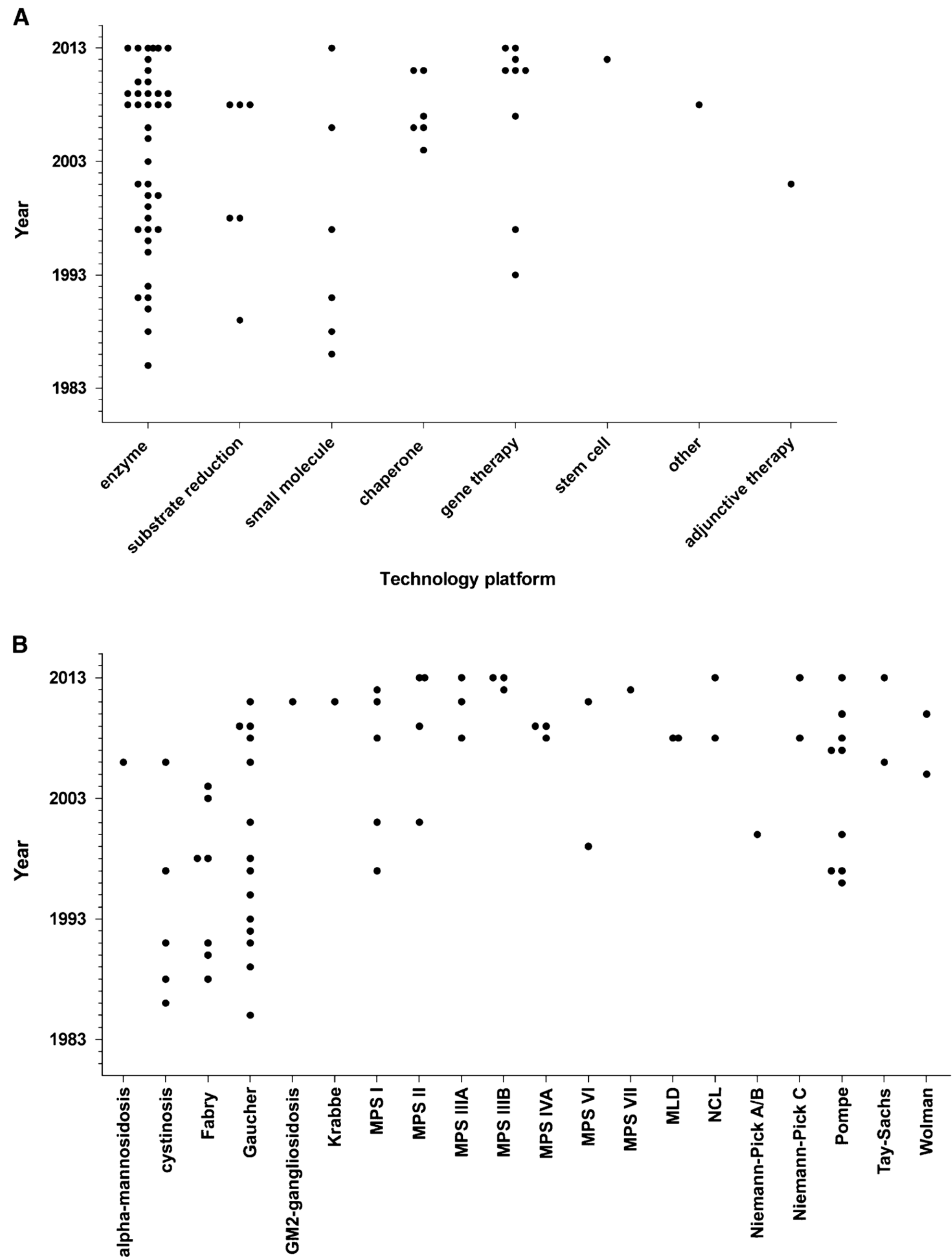

Fig. 4 A) Year of orphan drug designation for compounds intended to treat lysosomal storage disorders by technology platform. B) Year of orphan drug designation for compounds intended to treat lysosomal storage disorders by disease (Mechler et al 2015).

biosynthetic pathway and catalyzes the formation of glucocerebroside (Fig. 6) (Cox et al 2000). The drug approval was based on biomarker and visceral endpoints, i.e., on non-neurological measures. Small molecules are of particular interest for lysosomal storage disorders with neurological manifestations because they can cross the blood brain barrier. However, demonstrating the clinical effect can be challenging as illustrated by the results of a clinical trial with miglustat in Gaucher disease type 3 where effects on neurological endpoints could not be detected (Schiffmann et al 2008). More recently, another oral substrate inhibitor, eliglustat (Fig. 7), was approved for the treatment of Gaucher disease (Cox et al 2015; Mistry et al 2015). Eliglustat inhibits glucosylceramide synthase. It is different from miglustat as eliglustat resembles the ceramide rather than the glucose moiety and does not cross the blood–brain-barrier (Cox et al 2015).

Chemical chaperones enhance residual enzyme activity

Chemical chaperones are low molecular weight competitive enzyme inhibitors at high concentrations, but at very low concentrations they stabilize certain misfolded mutant enzymes

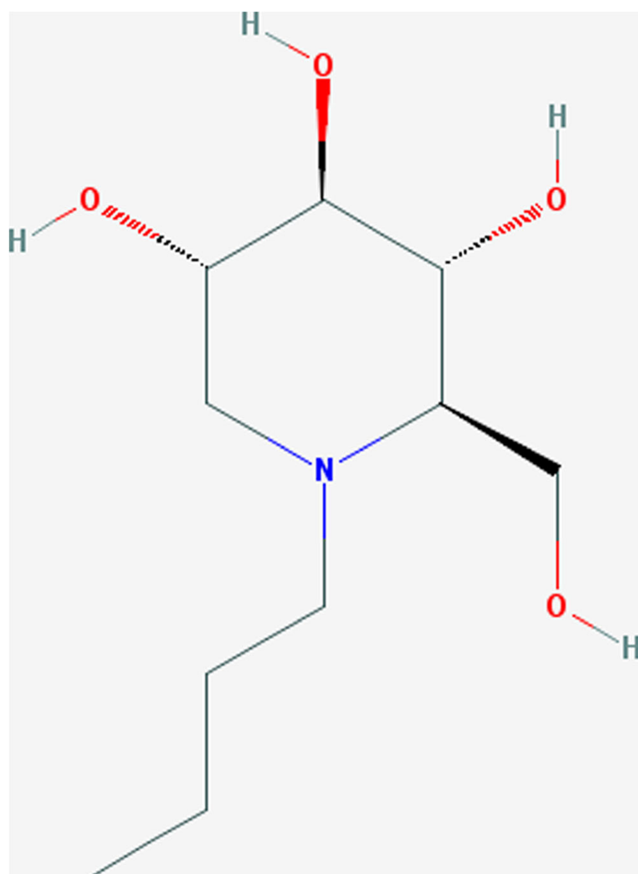

Fig. 5 Chemical structure of miglustat (NCBI 2017c)

(Fan et al 1999). Consecutively, these stabilized enzymes are protected from degradation in the endoplasmic reticulum/ Golgi system leaving the endoplasmic reticulum and are transported to the lysosome resulting in an augmentation of residual enzyme activity after dissociation of the chaperone

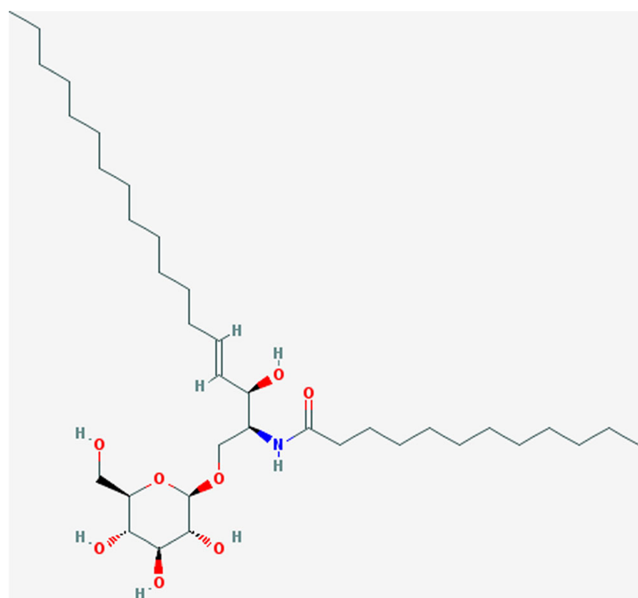

Fig. 6 Chemical structure of glucocerebroside (NCBI 2017a)

from the enzyme (Tropak et al 2004; Clarke et al 2011; Germain et al 2012; Khanna et al 2012; Sun et al 2012; Zimran et al 2013). Chemical chaperones are suitable only for a selected group of disease-causing missense mutations. Pathognomonic nonsense mutations would biochemically not be responsive, because the coded protein is truncated. As chemical chaperones can both inhibit and enhance enzyme activity depending on their concentrations in the local subcellular environment, the dose rationale in patients requires careful consideration. By 2013, six chemical chaperones have received orphan drug designation and clinical trials are ongoing (Mechler et al 2015). In 2016, migalastat was approved for treatment of Fabry disease in the EU, in the US the compound is investigated in further clinical trials (Germain et al 2016; Schiffmann and Ries 2016).

Small molecules facilitate substrate transport through subcellular compartments

Three small molecules, i.e., all derivatives of cysteamine, received orphan drug approval for the treatment of cystinosis, either applied systemically or topically in the eye (Gahl et al 1987; MacDonald et al 1990; Dohil et al 2010). In cystinosis, a cystine transporter gene mutation leads to an intralysosomal accumulation of cystine resulting among others in renal Fanconi syndrome and the formation of corneal crystals associated with photophobia. Cysteamine depletes cells from cystine by forming cysteine and cysteine-cysteamin complexes that can leave the lysosome. The most recent orphan drug approval for cystinosis was an extended release formulation of cysteamine that requires less frequent drug administration (BID instead of QID for the precurosor product) (Dohil et al 2010). In addition two cysteamine derivatives, i.e., phospohocysteamine and cyclodextrin, received orphan drug designation but not approval to date. Phosphocysteamine was intended to be developed as a formulation with higher bioavailability and better tolerance but the orphan drug status was withdrawn after pharmacokinetic studies in healthy volunteers did not confirm this hypothesis (Tenneze et al 1999). Cyclodextrin is a small molecule faciliating the intracellular transport of cholesterol in mouse models Niemann-Pick type C disease, another lysosomal transporter disease (Davidson et al 2009). Orphan drug designation was granted in 2013.

Bone-marrow derived multipotent stem cells and gene therapies have received FDA orphan drug designation status

A stem cell system—based on adult adherent bone marrow-derived multipotent stem cells—has received orphan drug designation for MPS I. These stem cells derived from a donor

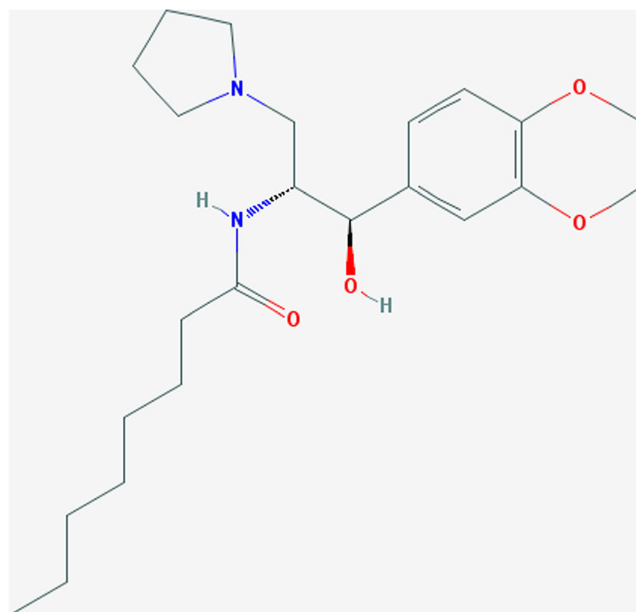

Fig. 7 Chemical structure of eliglustat (NCBI 2017b)

are subsequently expanded and scaled up in order to yield enough material to be used in a large number of patients. The concept was recently tested in MPS I mice that received bone marrow-derived multipotent progenitor cell cell transplants into the lateral cerebral ventricles (Nan et al 2012). Gene therapies have been granted orphan drug designation status for lysosomal storage disorders. Gene therapy is of particular interest in this context because there is the potential to continuously deliver enzyme into the organism rather than intermittent, thus, less physiological administration than in enzyme replacement therapy. Similar to chemical chaperones, these orphan drug designations were granted only recently (Fig. 4). The two older designations of 1993 and 1997 were based on a retroviral vector system. Designations after 2007 are based on adenovirus or lentivirus vectors (Dunbar et al 1998; Sun et al 2005; Ferla et al 2013; Haurigot et al 2013). One phase I/II clinical trial investigating adenovirus vector based gene therapy with a single intracerebral injection into both cerebral hemispheres in patients with MPS IIIA was conducted between 2011 and 2013 (clinicaltrials.gov identifier NCT 01474343).

The science of small clinical trials in lysosomal storage disorders and the next frontier, being able to treat the brain

In all lysosomal storage disorders pivotal trials leading to FDA or EMA approval were, by nature, small clinical trials (Table 1). In order to better understand inherent differences between *orphan* drug development and *traditional* drug development programs for more frequent diseases, the example of the rivaroxaban drug approval package is instructive and illustrative. Pivotal studies in the development program for rivaroxaban, an oral direct acting anticoagulant (factor Xa inhibitor), for the prophylaxis of deep vein thrombosis were conducted in three RCTs with a total of 9011 patients. Overall, the application of rivaroxaban was supported by 44 human clinical studies, eight population pharmacokinetic studies, 13 in vitro studies, and 16 biopharmaceutics studies (FDA 2011). These included human drug-drug-interaction studies, studies in populations with renal and hepatic impairment, a thorough QT interval prolongation study, and a food-effect study which are usually not part of an orphan drug development program. Pediatric studies were not conducted in the rivaroxaban program whereas a substantial proportion of patients in clinical registration trials for lysosomal storage disorders were children or adolescents. Of interest, none of the primary endpoints in the pivotal clinical trials for lysosomal storage disorders was purely neurological, although the majority of these diseases are associated with neurological involvement, such as neurodegeneration. The distance in a 6 minute walk test was a frequently used primary endpoint in registration trials for MPS I, MPS II, MPS VI (12 minute walk test), and Pompe disease (Table 1). The 6 minute walk test was initially developed and validated in patients with heart and lung diseases. Specifically, the test predicts morbidity and mortality in patients with chronic cardiopulmonary diseases such as primary pulmonary hypertension or advanced congestive heart failure (Miyamoto et al 2000; Shah et al 2001). Other genetic diseases with neuromuscular or pulmonary involvement, such as Duchenne muscular dystrophy or cystic fibrosis were also functionally assessed and quantified with the 6 minute walk test (Gulmans et al 1996; McDonald et al 2010).

Orphan drugs for lysosomal storage disorders were designated or developed either for mainly non-neurological and relatively more frequent conditions, or for diseases with a similar regulatory approach toward drug registration. Main pathophysiological biochemical pathways were known in all conditions with approved orphan drugs which renders lysosomal storage disorders interesting from a drug development perspective, because as exemplified in oncology, the understanding of molecular mechanisms of diseases is directly connected to the search for novel therapies (Stockklausner et al 2016). On the other hand, the precise mechanism of disease in lysosomal storage disorder is not fully understood, therefore, further research may provide insights into new therapeutic approaches. In addition, it would be desirable to have robust and unfragmented long-term outcome data for treatment in order to assess the potential for further innovation more thoroughly (Hollak et al 2011).

Currently available therapies show incomplete therapeutic response on advanced cardiac and skeletal manifestations and being able to treat brain disease is the next frontier. Drug development in neuronopathic lysosomal storage disorders

may be facilitated through the availability of instruments assessing neurological and behavioral functions in a standardized way (Gershon et al 2010). The journey may be long and challenging—first, approved orphan drugs are costly (Crow 2016). Second, as it took 30 years to develop drugs for seven lysosomal storage disorders and assuming that progress occurs at the same speed, by facing the obstacle of CNS bioavailabilty and smaller patient populations, it may still take many years of ambition and dedicated research to have one approved drug, for each of the 50 known lysosomal storage disorders today. Third, the availability of effective treatment may allow more and more LSDs to be added to newborn screening programs, and early treatment may subsequently improve outcome further.

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