FDA orphan drug designations for lysosomal storage disorders – a cross sectional analysis

Sven F. Garbade PhD 1, Matthias Zielonka MD 1, Konstantin Mechler MD 3, Stefan Kölker MD 1,
Georg F. Hoffmann MD 1, Christian Staunfer MD 1, Eugen Mengel MD 4 and Markus Ries MD PhD 1,2,5,*

Affiliations:

1 Division of Pediatric Neurology and Metabolic Medicine, Center for Pediatric and Adolescent Medicine, University Hospital Heidelberg, Heidelberg, Germany
2 Center for Rare Diseases, University Hospital Heidelberg, Heidelberg, Germany
3 Department of Child and Adolescent Psychiatry and Psychotherapy & Department of Addictive Behavior and Addiction Medicine, Central Institute of Mental Health, Medical Faculty Mannheim, University of Heidelberg, Mannheim, Germany
4 SphinCS GmbH, Science for LSD, Hochheim, Germany
5 Center for Virtual Patients, Medical Faculty, University of Heidelberg, Heidelberg, Germany

*Correspondence to:
Markus Ries, MD, PhD, MHSc, FCP
Division of Pediatric Neurology and Metabolic Medicine, Center for Pediatric and Adolescent Medicine, University Hospital Heidelberg
Im Neuenheimer Feld 430, D-69120 Heidelberg, Germany
Tel.: +49 6221 56 4002
markus.ries@uni-heidelberg.de

NOTE: This preprint reports new research that has not been certified by peer review and should not be used to guide clinical practice.
Declarations

Ethics approval and consent to participate: not applicable

Consent for publication: not applicable

Availability of data and material: all data generated or analysed during this study are included in this published article

Competing interests: SG, SK, and CS have no potential conflicts of interest to declare with respect to the research, authorship, and/or publication of this article. KM has served as investigator in clinical trials conducted by Emalex, Gedeon Richter, Lundbeck, Shire, Sunovion and Teva, plus in European Union funded projects. GFH received lecturing fees from Danone and Takeda. EM has received honoraria and/or consulting fees from Actelion, Alexion, BioMarin, Orphazyme, Sanofi Genzyme, and Shire. MR received consultancy fees or research grants from Alexion, GSK, Oxyrane and Shire.

Funding: no particular third-party funding was secured for this study.

Authors' contributions:

Substantial contributions to the conception or design of the work and supervision: MR

Data acquisition, analysis, interpretation of data: SG, MZ, KM, SK, GFH, CS, EM, MR

Drafting the work: MR

Substantively revision of the work: SG, MZ, KM, SK, GFH, CS, EM, MR

All authors have approved the submitted version (and any substantially modified version that involves the author's contribution to the study).

All authors have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.
Acknowledgements: Not applicable
Word count abstract: 227 words

Word count main text: 2108 words

Figures: 3

Tables: 2

Key words: orphan disease, orphan drug, drug development, lysosomal, FDA, enzyme, substrate reduction, gene therapy
Abstract

Purpose: To provide a quantitative clinical-regulatory insight into the status of FDA orphan drug designations for compounds intended to treat lysosomal storage disorders (LSD’s).

Methods: Assessment of the drug pipeline through analysis of the FDA database for orphan drug designations with descriptive and comparative statistics.

Results: Between 1983 and 2019, 124 orphan drug designations were granted by the FDA for compounds intended to treat 28 lysosomal storage diseases. Orphan drug designations focused on Gaucher disease (N=16), Pompe disease (N=16), Fabry disease (N=10), MPS II (N=10), MPS I (N=9), and MPS IIIA (N=9), and included enzyme replacement therapies, gene therapies, and small molecules, and others. Twenty-three orphan drugs were approved for the treatment of 11 LSDs. Gaucher disease (N=6), cystinosis (N=5), Pompe disease (N=3), and Fabry disease (N=2) had multiple approvals, CLN2, LAL-D, MPS I, II, IVA, VI, and VII one approval each. This is an increase of nine more approved drugs and four more treatable LSD’s (CLN2, MPS VII, LAL-D, and MPS IVA) since 2013. Mean time between orphan drug designation and FDA approval was 89.7 SD 55.00 (range 8-203, N=23) months.

Conclusions: The development pipeline is growing and evolving into diversified small molecules and gene therapy. CLN2 was the first and only LSD with an approved therapy directly targeted to the brain. Newly approved products included “me-too” – enzymes and innovative compounds such as the first pharmacological chaperone for the treatment of Fabry disease.
Introduction

Lysosomal storage disorders (LSDs) are a group of more than 50 inherited, multisystemic, progressive conditions caused by a genetic defect that results in the progressive accumulation of complex non-metabolized substrates in the lysosomes of cells, tissues and organs, inducing distinct but heterogeneous somatic and neurological disease phenotypes [1-7]. In general, lysosomal storage disorders lead to significant morbidity and decreased life expectancy. Reported prevalences of LSDs in industrialized countries range between 7.6 per 100,000 live births (=1 in 13,158) and 25 per 100,000 live births (=1 in 4000) [8-11]. Some LSDs are treatable and the drug development in the field has traditionally been very active and dynamic after the successful development of enzyme replacement therapy in Gaucher disease which seeded further innovation [1, 12, 13]. The development of new compounds and new concepts of treatment for lysosomal storage disorders has been very dynamic. Therefore, the purpose of the present paper is to precisely analyze the most recent advances and to document novel trends in orphan drug development for lysosomal storage diseases as documented in the FDA Orphan Drug Product designation database.
Methods

The FDA orphan drug database was accessed over the internet at the following address
http://www.accessdata.fda.gov/scripts/opdlisting/opod/. Search criteria were “all
designations” from 1 January 1983 until 10 May 2019, i.e., all data entries until 10 May 2019
were taken into account (N=4979). The output format was an excel file which was
downloaded on a local computer. Orphan designations for lysosomal storage diseases were
extracted with pertinent keywords. (N=124) [1]. STROBE criteria were respected [14].

Definitions

Pharmacological compounds were categorized based on their chemical structure into the
following classes, listed in alphabetical order: “enzyme”, “enzyme/small molecule
combination”, “gene therapy”, “polymer”, “protein (other than enzyme)”, and “small
molecule” [1]. A small molecule was defined as a compound with a molecular weight below
900 Da [15]. In addition, compounds were further grouped into functionally meaningfully
subtypes based on their biochemical properties, molecular mechanism of actions, or gene
therapy platforms, i.e., (in alphabetical order): “AAV vector”, “adjunctive therapy”,
“anaplerotic”, “anti-inflammatory/neuroprotective”, “anti-inflammatory/pro-chondrogenic”,
“anti-inflammatory/TPP1 enhancing”, “anti-inflammatory/TPP1 enhancing/vitamin
combination”, “pharmacological chaperone”, “cytochrom P450 rescue”, “enzyme
replacement therapy”, “enzyme replacement therapy –pharmacological chaperone co-
administration”, “lentiviral vector”, “membrane stabilization”, “lysosomal cholesterol
redistributor”, “replacement therapy with a modified enzyme”, “nonviral vector directing
transgene integration”, “receptor amplification”, “retroviral vector”, “small molecule
facilitating intracellular substrate transport”, “stem cells”, “stop codon read-through”,
“substrate reduction”.

7
Time to FDA approval was defined as the time period from orphan drug designation until approval by the FDA [1]. Drug approval rates were defined the proportion of orphan drug designations approved out of overall orphan drug designations granted. Missing data were not imputed.

Statistical analysis

Standard techniques of descriptive statistics were applied: continuous variables were summarized with mean, standard deviation, median, minimum and maximum values. Categorical variables were summarized with frequencies and percentages. Comparative statistics were performed with the appropriate parametric test for data with Gaussian distribution (i.e., ANOVA). Differences between frequency counts were compared with the chi-square test. Two-sided p-value $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SAS Enterprise guide 7.13 HF4, SAS Institute Inc., Cary, NC, USA. Graphs were generated with R [16] and GraphPad Prism 5.04, GraphPad Software, Inc., San Diego, CA, USA.
Results

The drug development pipeline: orphan drug designations granted by the FDA

Between 1 January 1983 and 10 May 2019, 124 orphan drug designations were granted by the FDA for compounds intended to treat 28 lysosomal storage diseases (Figure 1A). For a comparison of dimensions, in the same time period, the FDA granted 4979 orphan drug designations overall, out of which 783 were approved (Figure 1B). Twenty conditions had multiple orphan drug designations. Most orphan drug designations were granted for Gaucher disease (N=16), Pompe disease (N=16), Fabry disease (N=10), MPS II (N=10), MPS I (N=9), and MPS IIIA (N=9), followed by 14 others depicted in Figure 2A. Eight conditions had one orphan drug designation. Enzyme replacement therapies, gene therapies, small molecules, and other technology platform classes were designated as orphan drugs intended to treat lysosomal storage diseases (Figure 2B). Nine granted orphan drug designations were subsequently withdrawn (Table 1). The reason for withdrawal is not specified in the FDA orphan drug database.

The approval rate of lysosomal orphan drugs (18.5%) did not differ from approval rates for non-lysosomal orphan drugs (15.7%, p=0.38, chi-square)

Lysosomal storage disorders with FDA approved therapies

Twenty-three orphan drugs were approved for the treatment of 11 lysosomal storage diseases. Four diseases had multiple therapeutics approved, i.e. Gaucher disease (N=6), cystinosis (N=5), Pompe disease (N=3), and Fabry disease (N=2), (Figure 3A). The remaining seven diseases had one compound each approved by the FDA (i.e., CLN2, LAL-D, MPS I, II, IVA, VI, VII). CLN2 was the only neuronopathic lysosomal storage disease with an FDA approved therapy directly targeting the brain; all the other therapies address systemic non-neurological
manifestations. FDA approved therapies included enzyme replacement therapies (N=15) and small molecules (N=8), but no other class of drugs (Figure 3B, Table 2).

Drug development timelines

Overall mean time to approval, defined as time between orphan drug designation and FDA approval was 89.7 SD 55.00 (range 8-203, N=23) months. Stratified by drug compound subtypes, mean time to approval for enzyme replacement therapies was 81.2 SD 56.42 (range 8-203, N=15) months, mean time to approval for small molecules facilitating subcellular transport was 107.8 SD 52.96 (range 40-181, N=5) months, and mean time to approval for substrate reduction therapies was 66.5 SD 6.36 (range 62 to 71, N=2) months. Time to approval for the pharmacological chaperone therapy was 173 months. Differences between the groups were not statistically significant (p=0.33, ANOVA). The drug development timelines and market exclusivity periods, an incentive granted by the FDA to stimulate orphan drug development [13], are illustrated in Figure 3A.
Discussion

By 10 May 2019, 23 orphan drugs were approved by the FDA for the treatment of 11 lysosomal storage disorders. This is an increase of nine more approved orphan drugs and four more treatable lysosomal disease (i.e. CLN2, MPS VII, LAL-D, and MPS IVA) compared to 2013 [1].

While alglucerase for Gaucher disease was the first orphan drug approved for a lysosomal storage disease in 1991, intrathecally administered cerliponase alfa for CLN2, FDA approved in 2017, is the first orphan drug approved to directly treat the brain which is a significant therapeutic innovation [17, 18]. Since 2013, 54 more orphan drug designations were granted. In addition, diseases such as CLN1, CLN3, CLN4, Farber disease, and GM1-gangliosidosis did not have orphan drug designations in 2013, which indicates that drug development in lysosomal storage disorders is now being driven into mainly neuronopathic conditions (Figure 2A). The overall growth curve of orphan drug designations appears to accelerate over time and may become exponential (Figure 1A), which may follow indeed a global trend (Figure 1B). Of interest, the drug approval rate in lysosomal orphan drug development and non-lysosomal orphan drug development did not differ. Technology is evolving: while enzyme replacement therapies had initially set the trend, more modified enzymes, including fusion-proteins, and an enzyme-chaperone co-administration entered the development pipeline. This may be a reaction to the increasing recognition in the field, that, in general, systemically administered enzyme replacement therapy with conventional enzymes can easily access organs such as liver and spleen, but have little impact on bone and CNS manifestations. Four small molecules have been approved by the FDA for the treatment of a lysosomal storage disease. Their mechanisms of action target the facilitation of subcellular transport (e.g., cysteamine for cystinosis, approved in 1994), and the reduction of storage (miglustat, approved in 2003, and eliglustat, approved in 2014, both for the treatment of Gaucher disease)
In 2018, migalastat, which stabilizes the misfolded enzyme alpha-galactosidase A, was approved as first-of-its kind pharmacological chaperone by the FDA for the treatment of Fabry disease [19], (Table 2). Mechanisms of action for small molecules, either approved or in drug development, considered the broad spectrum of underlying pathophysiology and aimed at 1) targeting the affected gene 2) targeting the affected enzyme 3) targeting storage 4) targeting cellular uptake of therapeutic enzymes, and 4) mitigation of cellular damage or anaplerotic (Table 2). It is possible and likely that not all mechanistically meaningful approaches lead to clinical benefit in patients [20]. The plethora of innovative ideas for pharmacological approaches is laudable, but should not lead a treating physician to engage in off-label use, but rather encourage international collaboration aimed to generate the highest standard of evidence-based knowledge by respecting excellence clinical research [21].

Gene therapy now plays a larger role in the drug development pipeline compared to the situation in our last analysis [22]. This may again be a reaction to the increased recognition of enzyme replacement therapies’ substantial limitations, as described in detail above. The technical approach towards gene therapy is evolving as illustrated in Figure 2B. Gene therapies rely at least in principle on the assumption that a single treatment may result in a sustained, potentially curative clinical benefit for the patients. The first molecular tools enabling efficient non-toxic gene transfer into human somatic cells were recombinant replication-deficient vectors [23]. Among those, retroviral and adeno-associated viral (AAV) vectors have been the most widely used in particular for ex vivo T cell engineering or genetically engineered hematopoietic stem cells (HSCs) for the treatment of primarily hematologic or oncologic conditions such as pediatric ALL, β-thalassemia or adenosine deaminase deficiency [24-26]. In contrast, while the first two orphan drug designations for gene therapy for lysosomal storage diseases in 1993 and 1997 (both for Gaucher disease) relied on retroviral vectors, this platform was subsequently abandoned. This is likely due to the emergence of serious toxicities related to high gene transfer including insertional
genotoxicity, immune destruction of genetically modified cells, and immune reactions related to the application of certain vectors [27, 28]. The next step in gene technology was the introduction of AAV (designated for Pompe disease in 2007), followed by stop-codon read through (designated in 2014 and 2016 for MPS I, and in 2018 for cystinosis). Moreover, lentiviral vector (designated in 2018 for Fabry disease and MLD), and nonviral vector directing transgene integration (designated in 2018 for MPS I) technologies are being considered, all of which have to prove their safety and efficacy in the future. More sophisticated genome editing technologies that enable a variety of therapeutic genome modifications (gene addition, gene ablation or “gene correction”) consist of the administration of transcription activator-like effector nucleases (TALENs) and or CRISPR-Cas 9 system to efficiently cleave and modify DNA at sites of interest [29-33]. Those approaches are currently limited to applications in basic research, but transfer into clinical trials can be expected in the near future [34, 35]. Until close of database no gene therapy was approved for the treatment of lysosomal storage disorders (Figure 3B). If proven successful in registration trials - which would supposedly be small clinical trials of a limited duration - it is of particular interest, how long the therapeutic effect of gene therapy can be sustained during a patients’ lifetime, and, if this time is limited, whether it would be safe and feasible to repeat the administration of a gene therapy multiple times in a single patient. It is anticipated that novel therapies will be costly. Important topics for future investigations will include patient selection, starting and stopping criteria.

For the correct contextual interpretation it is important to be aware of important limitations of the present work as pointed out previously [1]. Orphan drug designation was considered as the expressed intent to develop a drug. This may be biased by strategic and patent related considerations and not all manufacturers may choose to go this publically visible pathway from early on. Time of orphan drug designation may be somewhat arbitrary in the drug
development process; therefore, time-to-approval as presented in the present analysis may also be biased by the intellectual property strategy of the respective drug development program. Orphan drug development outputs in jurisdictions other than the FDA were, similar to our previous analysis, not taken into account because this analysis was by definition focused on the impact of the US orphan drug act [1, 13]. As drug development in lysosomal storage disorders is, in general, a global enterprise we consider the present finding within the context of their limitations generalizable.

Conclusions
Activities in orphan drug development for lysosomal storage disorders are steadily increasing which follows a global trend in orphan drug development overall. Newly approved products included “me-too” – enzymes, but also innovative compounds such as the first ERT targeting the brain in CLN2 and the first-of-its-kind pharmacological chaperone for the treatment of Fabry disease. The development pipeline is increasingly evolving into diversified small molecules and, in particular, gene therapies.
Table 1: Withdrawn orphan drug designations. Reasons for and time of withdrawal were not specified in the FDA database

| Compound                        | Pharmacological subtype | Year of orphan drug designation | Indication under development                                      |
|---------------------------------|-------------------------|----------------------------------|---------------------------------------------------------------------|
| Ataluren                        | Stop codon read-through | 2014                             | Treatment of mucopolysaccharidosis type I                           |
| Recombinant human alpha-N-acetylglucosaminidase | Enzyme                  | 2013                             | Treatment of mucopolysaccharidosis IIIB (Sanfilippo B syndrome)     |
| Recombinant human arylsulphatase A | Enzyme                  | 2008                             | Treatment of metachromatic leukodystrophy (MLD)                     |
| Miglustat                       | Substrate reduction     | 2008                             | Treatment of the neurological manifestations of Niemann-Pick disease, type C |
| Duvoglustat hydrochloride        | Substrate reduction     | 2007                             | Treatment of Pompe disease                                         |
| Isofagomine tartrate | Chaperone | 2006 | Treatment of Gaucher disease |
|----------------------|-----------|------|-----------------------------|
| Retroviral vector, R-GC and GC gene | Retroviral vector | 1997 | Treatment of Gaucher disease |
| Human acid precursor alpha-glucosidase, recombinant | Enzyme | 1996 | Treatment of glycogen storage disease type II |
| Phosphocysteamine | Substrate reduction | 1988 | Treatment of cystinosis. |
Table 2. Mechanism of action of FDA approved small molecules (*) and small molecules in development, intended to treat a lysosomal storage disorder.

| Mechanism of action | Compound | Disease with FDA orphan drug designation |
|---------------------|----------|------------------------------------------|
| **Targeting the affected gene:** | | |
| Stop codon read through in missense mutations | 6‘-(R)-methyl-5-O-(5-amino-5,6-dideoxy-alpha-L-talofuranosyl)-paromamine sulfate | MPS I Cystinosis |
| | Gemfibrozil | CLN |
| | N-t-butylhydroxylamine | CLN1 |
| | Modified cholera toxin | Gaucher disease |
| | Pyrimethamine | GM2-gangliosidosis |
| Drug/Compound | Disease |
|---------------|---------|
| Ambroxol      | Gaucher disease |
| N-acetyl-glucosamine thiazoline | Adult Tay-Sachs disease |
| Migalastat hydrochloride* | Fabry disease |
| Recombinant human acid α-glucosidase/miglustat | Pompe disease |
| Odiparcil     | MPS VI |
| Lucerastat    | Fabry disease |
| Venglustat    | Fabry disease |
| (3S)-1-azabicyclo[2.2.2]oct-3-yl{2-[2-(4-fluorophenyl)-1,3-thiazol-4-yl]propan-2-yl}carbamate | Gaucher disease |
| 2-hydroxypropyl-B-cyclodextrin§ | Niemann-Pick disease type C |

**Targeting storage:**
Substrate reduction and subcellular storage redistribution

[43-45]
| Drug                                      | Disease/Condition               |
|-------------------------------------------|---------------------------------|
| Hydroxy-Propyl-Beta-Cyclodextrin§         | Niemann-Pick disease type C     |
| Miglustat*                                | Gaucher disease*                |
| Eliglustat*                               | Gaucher disease type I*         |
| Cysteamine*                               | Cystinosis*                     |
| 1,5-(Butylimino)-1,5 dideoxy,D-glucitol   | Fabry disease                   |
| L-cycloserine                             | Gaucher disease                 |
| 1,5-(Butylimino)-1,5 dideoxy,D-glucitol   | Fabry disease                   |

**Targeting cellular uptake of therapeutic enzymes:**
- Receptor amplification [46]

**Mitigation of cellular damage**
- (Antiinflammatory, prochondrogenic, neuroprotective cytochrome P450 rescue) or anaplerotic [20, 37, 47-49]

| Drug                                      | Disease/Condition               |
|-------------------------------------------|---------------------------------|
| Ursodeoxycholic acid                      | Niemann-Pick C                  |
| Gemfibrozil and vitamin A                 | CLN                             |
|                        |                        |
|------------------------|------------------------|
| Ibudilast              | Krabbe disease         |
| Pentosan polysulfate sodium | MPS VI                  |
| Triheptanoin           | Pompe disease          |

§protein acting as a chaperone

§polymer
Figures

**Figure 1A:** Number of orphan drug designations (open bars) and FDA approvals (full bars) for compounds intended to treat lysosomal storage diseases by year.

* indicates close of database: 10 May 2019

![Graph showing orphan designations and FDA approvals by year for lysosomal storage diseases](image-url)
Figure 1B: Overall number of orphan drug designations (open bars) and FDA approvals (full bars) by year.

* indicates close of database: 10 May 2019
**Figure 2A:** Orphan drug designations granted by the FDA for compounds intended to treat lysosomal storage disorders by year and specific disease.
Figure 2B: Orphan drug designations granted by the FDA for compounds intended to treat lysosomal storage disorders by year and pharmacological technology platform.
Figure 3A: FDA approved compounds for the treatment of lysosomal storage disorders (depicted as compound#disease), development times and market exclusivity. Grey bars indicate drug development times, i.e. time from orphan drug designation to orphan drug approval by the FDA. Black bars indicate, if applicable, market exclusivity periods.

Treatments for cystinosis included three different formulations (systemic, systemic extended release, and ophthalmic), and three different age groups (adults, children 2-6 years, and children 1 year of age to less than 2 years of age.)
Figure 3B: FDA approved therapies for the treatment of lysosomal storage disorders by year of approval and pharmacological technology platform.
Literature

1. Mechler K, Mountford WK, Hoffmann GF, Ries M: Pressure for drug development in lysosomal storage disorders - a quantitative analysis thirty years beyond the US orphan drug act. *Orphanet J Rare Dis* 2015, 10:46.
2. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M: Quantitative clinical characteristics of 53 patients with MPS VII: a cross-sectional analysis. *Genet Med* 2017, 19:983-988.
3. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M: A cross-sectional quantitative analysis of the natural history of Farber disease: an ultra-orphan condition with rheumatologic and neurological cardinal disease features. *Genet Med* 2018, 20:524-530.
4. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M: A cross-sectional quantitative analysis of the natural history of free sialic acid storage disease-an ultra-orphan multisystemic lysosomal storage disorder. *Genet Med* 2018.
5. Slama T, Garbade SF, Kolker S, Hoffmann GF, Ries M: Quantitative natural history characterization in a cohort of 142 published cases of patients with galactosialidosis-A cross-sectional study. *J Inherit Metab Dis* 2019, 42:295-302.
6. Komatsuzaki S, Zielonka M, Mountford WK, Kolker S, Hoffmann GF, Garbade SF, Ries M: Clinical characteristics of 248 patients with Krabbe disease: quantitative natural history modeling based on published cases. *Genet Med* 2019.
7. Zielonka M, Garbade SF, Kolker S, Hoffmann GF, Ries M: Ultra-orphan lysosomal storage diseases: A cross-sectional quantitative analysis of the natural history of alpha-mannosidosis. *J Inherit Metab Dis* 2019.
8. Meikle PJ, Hopwood JJ, Clague AE, Carey WF: Prevalence of lysosomal storage disorders. *JAMA* 1999, 281:249-254.
9. Poorthuis BJ, Wevers RA, Kleijer WJ, Groener JE, de Jong JG, van Weely S, Niezen-Koning KE, van Diggelen OP: The frequency of lysosomal storage diseases in The Netherlands. *Hum Genet* 1999, 105:151-156.
10. Applegarth DA, Toone JR, Lowry RB: Incidence of inborn errors of metabolism in British Columbia, 1969-1996. *Pediatrics* 2000, 105:e10.
11. Pinto R, Caseiro C, Lemos M, Lopes L, Fontes A, Ribeiro H, Pinto E, Rocha S, Marcão A, et al: Prevalence of lysosomal storage diseases in Portugal. *Eur J Hum Genet* 2004, 12:87-92.
12. Ries M: Enzyme replacement therapy and beyond—in memoriam Roscoe O. Brady, M.D. (1923-2016). *J Inherit Metab Dis* 2017, 40:343-356.
13. Public Law 97-414, 97th Congress, An Act To amend the Federal Food, Drug, and Cosmetic Act to facilitate the development of drugs for rare diseases and conditions, and for other purposes [https://www.fda.gov/media/99546/download](https://www.fda.gov/media/99546/download).
14. Vandenbroucke JP, von Elm E, Altman DG, Gotzsche PC, Mulrow CD, Poole C, Schlesselman JJ, Egger M, Initiative S: Strengthening the Reporting of Observational Studies in Epidemiology (STROBE): explanation and elaboration. *PLoS Med* 2007, 4:e297.
15. Macielag MJ: Chemical properties of antibacterials and their uniqueness. In *Antibiotic Discovery and Development*. Edited by Dougherty TJ, Pucci MJ. New York Springer Science+Business Media, LLC; 2012: 793-820.
16. R Core Team: *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria.; 2019.
17. Barton NW, Brady RO, Dambrosia JM, Di Bisceglie AM, Doppelt SH, Hill SC, Mankin HJ, Murray GJ, Parker RI, Argoiff CE, et al.: Replacement therapy for inherited enzyme deficiency--macrophage-targeted glucocerebrosidase for Gaucher's disease. *N Engl J Med* 1991, 324:1464-1470.
18. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, Dyke JP, Cahan H, Slasor P, Jacoby D, et al: *Study of Intraventricular Cerliponase Alfa for CLN2 Disease*. *N Engl J Med* 2018, 378:1898-1907.
19. Germain DP, Hughes DA, Nicholls K, Bichet DG, Giugliani R, Wilcox WR, Feliciani C, Shankar SP, Ezgu F, Amartino H, et al: Treatment of Fabry's Disease with the Pharmacologic Chaperone Migalastat. *N Engl J Med* 2016, 375:545-555.
20. Schiffmann R, Wallace ME, Rinaldi D, Ledoux I, Luton MP, Coleman S, Akman HO, Martin K, Hogrel JY, Blankenship D, et al: A double-blind, placebo-controlled trial of triheptanoin in adult polyglucosan body disease and open-label, long-term outcome. *J Inherit Metab Dis* 2018, 41:877-883.
21. Lampert A, Hoffmann GF, Ries M: Ten Years after the International Committee of Medical Journal Editors’ Clinical Trial Registration Initiative, One Quarter of Phase 3 Pediatric Epilepsy Clinical Trials Still Remain Unpublished: A Cross Sectional Analysis. *PLoS One* 2016, 11:e0144973.

22. Nagree MS, Scalia S, McKillop WM, Medin JA: An update on gene therapy for lysosomal storage disorders. *Expert Opin Biol Ther* 2019, 19:655-670.

23. Kotterman MA, Chalberg TW, Schaffer DV: *Viral Vectors for Gene Therapy: Translational and Clinical Outlook*. *Annu Rev Biomed Eng* 2015, 17:63-89.

24. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Chew A, Gonzalez VE, Zheng Z, Lacey SF, et al: Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med* 2014, 371:1507-1517.

25. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, et al: Transfusion independence and HMG2A2 activation after gene therapy of human beta-thalassaemia. *Nature* 2010, 467:318-322.

26. Aiuti A, Cattaneo F, Galimberti S, Benninghoff U, Cassiani I, Callegaro L, Scaramuzza S, Andolfi G, Mirolo M, Brigida I, et al: Gene therapy for immunodeficiency due to adenosine deaminase deficiency. *N Engl J Med* 2009, 360:447-458.

27. Jenks S: *Gene therapy death--“everyone has to share in the guilt”*. *J Natl Cancer Inst* 2000, 92:98-100.

28. Nienhuis AW, Dunbar CE, Sorrentino BP: Genotoxicity of retroviral integration in hematopoietic cells. *Mol Ther* 2006, 13:1031-1049.

29. Li T, Huang S, Jiang WZ, Wright D, Spalding MH, Weeks DP, Yang B: TAL nucleases (TALNs): hybrid proteins composed of TAL effectors and FokI DNA-cleavage domain. *Nucleic Acids Res* 2011, 39:359-372.

30. Port F, Bullock SL: Creating Heritable Mutations in Drosophila with CRISPR-Cas9. *Methods Mol Biol* 2016, 1478:145-160.

31. Reyon D, Tsai SQ, Khayter C, Foden JA, Sander JD, Joung JK: FLASH assembly of TALENs for high-throughput genome editing. *Nat Biotechnol* 2012, 30:460-465.

32. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM: RNA-guided human genome engineering via Cas9. *Science* 2013, 339:823-826.

33. Doudbu JA, Charpentier E: Genome editing. The new frontier of genome engineering with CRISPR-Cas9. *Science* 2014, 346:1258096.

34. Joung JK, Sander JD: TALENs: a widely applicable technology for targeted genome editing. *Nat Rev Mol Cell Biol* 2013, 14:49-55.

35. Komor AC, Badran AH, Liu DR: CRISPR-Based Technologies for the Manipulation of Eukaryotic Genomes. *Cell* 2017, 168:20-36.

36. Leubitz A, Frydman-Marom A, Sharpe N, van Duzer J, Campbell KCM, Vanhoutte F: Safety, Tolerability, and Pharmacokinetics of Single Ascending Doses of ELX-02, a Potential Treatment for Genetic Disorders Caused by Nonsense Mutations, in Healthy Volunteers. *Clin Pharmacol Drug Dev* 2019.

37. Kim K, Kleinnan HK, Lee HJ, Pahan K: Safety and potential efficacy of gemfibrozil as a supportive treatment for children with late infantile neuronal ceroid lipofuscinoses and other lipid storage disorders. *Orphanet J Rare Dis* 2017, 12:113.

38. Adhan H, Zhang Z, Park HJ, Tailor C, Che C, Kami M, Spitalny G, Binnington B, Lingwood C: Endoplasmic Reticulum-Targeted Subunit Toxins Provide a New Approach to Rescue Misfolded Mutant Proteins and Revert Cell Models of Genetic Diseases. *PLoS One* 2016, 11:e0166948.

39. Parenti G, Fecarotta S, la Marca G, Rossi B, Ascione S, Donati MA, Morandi LO, Raviglia S, Pichiecchio A, Ombrone D, et al: A chaperone enhances blood alpha-glucosidase activity in Pompe disease patients treated with enzyme replacement therapy. *Mol Ther* 2014, 22:2004-2012.

40. Enshaei H, Molina BG, Del Valle LJ, Estrany F, Arnan C, Puiggali J, Saperas N, Aleman C: Scaffolds for Sustained Release of Ambroxol Hydrochloride, a Pharmacological Chaperone That Increases the Activity of Misfolded beta-Glucocerebrosidase. *Macromol Biosci* 2019:e1900130.

41. Osher E, Fattal-Valevski A, Sagie L, Urshanski N, Sagiv N, Peleg L, Lerman-Sagie T, Zimran A, Elstein D, Navon R, et al: Effect of cyclic, low dose pyrimethamine treatment in patients with Late Onset Tay Sachs: an open label, extended pilot study. *Orphanet J Rare Dis* 2015, 10:45.

42. Valenzano KJ, Khanna R, Powe AC, Boyd R, Lee G, Flanagan JJ, Benjamin ER: Identification and characterization of pharmacological chaperones to correct enzyme deficiencies in lysosomal storage disorders. *Assay Drug Dev Technol* 2011, 9:213-235.

43. Guerard N, Oder D, Nordbeck P, Zwingelstein C, Morand O, Welford RWD, Dingemans J, Wanner C: Lucerastat, an Iminosugar for Substrate Reduction Therapy: Tolerability, Pharmacodynamics,
and Pharmacokinetics in Patients With Fabry Disease on Enzyme Replacement. Clin Pharmacol Ther 2018, 103:703-711.

44. Entchev E, Jantzen I, Gawronski X, Feraille L, Luccarini J, Abitbol J, Junien J, Broqua P, Tallandier M: Odiparcil is a promising substrate reduction therapy in MPS VI murine model. Mol Genet Metab 2018, 123:S42-S43.

45. Ory DS, Ottinger EA, Farhat NY, King KA, Jiang X, Weissfeld L, Berry-Kravis E, Davidson CD, Bianconi S, Keener LA, et al: Intrathecal 2-hydroxypropyl-beta-cyclodextrin decreases neurological disease progression in Niemann-Pick disease, type C1: a non-randomised, open-label, phase 1-2 trial. Lancet 2017, 390:1758-1768.

46. Koeberl DD, Case LE, Smith EC, Walters C, Han SO, Li Y, Chen W, Hornik CP, Huffman KM, Kraus WE, et al: Correction of Biochemical Abnormalities and Improved Muscle Function in a Phase I/II Clinical Trial of Clenbuterol in Pompe Disease. Mol Ther 2018, 26:2304-2314.

47. Bongarzone ER, Escolar ML, Gray SJ, Kafri T, Vite CH, Sands MS: Insights into the Pathogenesis and Treatment of Krabbe Disease. Pediatr Endocrinol Rev 2016, 13 Suppl 1:689-696.

48. Schuchman EH, Ge Y, Lai A, Borisov Y, Faillace M, Eliyahu E, He X, Iatridis J, Vlassara H, Striker G, Simonaro CM: Pentosan polysulfate: a novel therapy for the mucopolysaccharidoses. PLoS One 2013, 8:e54459.

49. Roe CR, Mochel F: Anaplerotic diet therapy in inherited metabolic disease: therapeutic potential. J Inherit Metab Dis 2006, 29:332-340.