Silva *et al. Rare Dis Orphan Drugs J* 2022;1:13
DOI: 10.20517/rdodj.2022.09

Rare Disease and Orphan Drugs Journal

Editorial

Open Access

Check for updates

Enzyme replacement therapy: current challenges and drug delivery prospects via extracellular vesicles

Amanda K. A. Silva[1], Corinne Sagné[2], Florence Gazeau[1], Ibane Abasolo[3,4]

[1]Laboratoire MSC Matière et Systèmes Complexes, Université Paris Cité, CNRS UMR 7057, Paris 75006, France.
[2]Saints-Pères Paris Institute for the Neurosciences, Université Paris Cité, CNRS UMR 8003, Paris 75006, France.
[3]Drug Delivery & Targeting (DDT) and Functional Validation & Preclinical Research (FVPR), Vall d'Hebron Institute of Research (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona 08035, Spain.
[4]Networking Research Center on Bioengineering Biomaterials and Nanomedicine (CIBER-BBN), Barcelona 08035, Spain.

Correspondence to: Dr. Amanda K A Silva, Laboratoire MSC Matière et Systèmes Complexes, Université Paris Cité, CNRS UMR 7057, Paris 75006, France. E-mail: amanda.silva@univ-paris-diderot.fr; Dr. Ibane Abasolo, Drug Delivery & Targeting (DDT) and Functional Validation & Preclinical Research (FVPR), Vall d'Hebron Institute of Research (VHIR), Universitat Autònoma de Barcelona (UAB), Barcelona 08035, Spain and Networking Research Center on Bioengineering Biomaterials and Nanomedicine (CIBER-BBN), Barcelona 08035, Spain. E-mail: ibane.abasolo@vhir.org

How to cite this article: Silva AKA, Sagné C, Gazeau F, Abasolo I. Enzyme replacement therapy: current challenges and drug delivery prospects via extracellular vesicles. *Rare Dis Orphan Drugs J* 2022;1:13. https://dx.doi.org/10.20517/rdodj.2022.09

Received: 23 Jun 2022 **Accepted:** 28 Jun 2022 **Published:** 4 Jul 2022

Academic Editor: Daniel Scherman **Copy Editor:** Jia-Xin Zhang **Production Editor:** Jia-Xin Zhang

Lysosomal storage disorders (LSDs) comprise > 70 inborn errors of metabolism that are individually considered rare diseases but all together concern 1 in 5000 live births[1]. LSDs are caused by defects in genes related to the lysosomal homeostasis. This results in the abnormal storage of macromolecules in cells, triggering chronic inflammation[2]. About 70% of LSDs feature neurodegenerative damage[3]. Therapeutic strategies comprise gene therapy, therapies based on small molecules (i.e., chaperones), organ/cell transplantation (bone marrow transplantation), substrate reduction therapy, and enzyme replacement therapy (ERT)[4]. At present, the most important clinically approved therapy for several LSDs is ERT[5].

ERT is based on the periodic life-long intravenous administration of specific enzymes purified from cells engineered for their specific production with recombinant DNA technology, to palliate impaired enzyme function in patients[5,6]. Since the first ERT was clinically approved for Gaucher disease (β-

glucocerebrosidase deficiency) in 1991, several other enzymes have been approved for clinical use [Table 1]. ERT is a costly therapy reaching about $200,000/year[4].

ERT's current challenges are multiple. They include the following.

Safety and efficacy: An important issue concerning ERT relates to the immune response exerted against these biologicals. This issue was highlighted by the FDA, indicating the need for investigations on monitoring tests and therapy optimization[11]. The neutralizing antibodies can reduce the therapeutic outcome of ERTs as they can bind to the enzyme active site and/or ligands involved in the interaction with a receptor on the cells of interest, blocking the cellular uptake[12]. Therefore, patient immune response to the frequent enzyme administration ultimately affects ERT safety and/or treatment efficacy. This is especially relevant in the case of Pompe disease, as newborns developing antibodies do not respond to treatment and have no other treatment option.

Tissue targeting: Reaching the central nervous system (CNS) is one of the main challenges of ERT in order to provide effective therapy for most of the disease conditions. Indeed, recombinant enzymes infused intravenously fail to cross the blood-brain barrier (BBB) and access the CNS[13]. Large, polar molecules such as the recombinant enzymes used for ERT are poorly amenable to cross the BBB[14] and enzymes engineered to display an increased brain endothelium transcytosis by fusion with a monoclonal antibody directed against a brain endothelium receptor, e.g., insulin or transferrin receptors, are currently under clinical investigation for MPS[15]. ERTs are clinically approved for very few diseases [Table 1]. For the clinically approved ERTs, the main targets are peripheral sites. Once in the circulation, the administered enzymes feature a short half-life. The major proportion of the administered recombinant enzymes is distributed to the visceral organs[5,6].

Cellular targeting: Enzyme delivery to its cellular and/or subcellular (lysosomal) targets is an additional challenge. In the case the enzymes are insufficiently or not properly glycosylated, there may be an impairment in their ability to bind receptors, enter the cells, and reach their lysosomal target. These post-translational modifications are a limiting factor in the production of enzymes for ERT[4]. Additionally, the high expression levels of the mannose receptor in the liver also render difficult ERT delivery to tissues where it is the most needed such as macrophages in Gaucher disease[16].

Restriction to soluble enzymes: ERT based on the infusion of a purified enzyme is not relevant for LSDs arising from mutations in lysosomal membrane proteins such as CLN3 because large-scale purification of active membrane proteins is technically challenging and requires a lipidic or lipid-like environment.

ERT may fail to provide the expected therapeutic effect considering not only its non-specific biodistribution but also its low bioavailability due to a high biodegradation rate[17]. There is a real need in ERT for the design of targeted delivery systems combining both efficacy and safety to protect and address enzymes.

Enzyme encapsulation in nanosystems can overcome key challenges in ERT, including undesired immunologic reactions and biodegradation. It can also prevent non-selective biodistribution and improve the pharmacological response by increasing the drug absorption, enzyme controlled release, pharmacokinetics, and pharmacodynamics properties[17]. Current ERT nanomedicine research has focused on polymeric nanoparticles[18], liposomes[19], and other platforms such as protein aggresomes[20]. The liposomal strategy was reported by Cabrera *et al.* for Fabry disease[21] and by others for Batten disease[19,22]. More recently, extracellular vesicles (EVs) have been proposed as a bio-platform for ERT delivery for

Table 1. Enzyme replacement therapy clinically available or under evaluation (updated from Refs.[4,7-10])

Disease (deficient enzyme)	ERT	Approval for clinical use (year/regulatory agency)
Gaucher's disease (β-glucoce-rebrosidase)	Velaglucerase (VPRIV™)	2010-FDA, EMA; 2014-PMDA
	Taliglucerase (Elelyso™)	2012-FDA
	Imiglucerase (Cerezyme®)	FDA-1991, EMA-1997
Fabry disease (α-galactosidase)	Agalsidase α (Fabrazyme™)	2001-EMA
	Agalsidase β (Replagal™)	2003-FDA, EMA
	PRX-102 (pegunigalsidase alfa)	Submited in 2022 to EMA
Pompe disease (α-glucosidase)	Aglucosidase (Myozyme™)	2006-FDA, EMA, PMDA
	Aglucosidase (Lumizyme™)	2010-FDA
	Avalglucosidase alfa (Nexviazyme®)	EMA-2021, FDA-2021
MPS I (Hurler syn.) (α-L-iduronidase)	Laronidase (Aldurazyme™)	2003-FDA, EMA; 2006-PMDA
MPS II (Hunter syn.) Iduronate-2-sulfatase	Idursulfase (Elaprase™)	2006-FDA; 2007-EMA, PMDA
MPS IV A (Morquio A syn.) (N-acetylgalactosamine 6-sulfatase)	Elosulfase Alfa (Vimzim™)	2014-FDA
MPS VI (Maroteaux-Lamy syn.) (arylsulfatase B)	Galsulfase (Naglazyme™)	2005-FDA; 2006-EMA; 2008-PMDA
Lysosomal acid lipase deficiency (Lysosomal acid lipase)	Sebelipase α (Kanuma™)	2015-FDA, EMA; 2016-PMDA
CLN2 defficiency -> Brineura (2017)	cerliponase alpha (Brineura®)	2017-FDA and EMA
mucopolysaccharidosis type VII (MPS VII)	Vestronidase alfa (Mepsevii™)	2017-FDA and EMA-2018
Gaucher disease (β-glucocerebrosidase deficiency)	Imiglucerase (Cerezyme®)	FDA-1991, EMA-1997

FDA: Food and Drug Administration; EMA: European Medicines Agency; PMDA: Pharmaceuticals and Medical Devices Agency.

Gaucher disease[23,24]. EVs, encompassing exosomes, microvesicles, and apoptotic bodies, are sub-cellular entities (40-5000 nm diameter) secreted by cells in a constitutive or inducible manner[25-27]. Importantly, more than conferring enzyme protection, EVs may enable enzymes to reach the CNS as EVs are able to cross the BBB[28,29].

Seras-Franzoso *et al.* reported the feasibility of EVs for both Fabry and Sanfilippo diseases. They demonstrated that EVs released by CHO DG44 and HEK293 cells transfected with plasmids coding for alpha-galactosidase A (GLA) or N-sulfoglucosamine sulfohydrolase (SGSH) enzymes (defective in Fabry and Sanfilippo A diseases, respectively) were constitutively loaded with these enzymes[30]. This outstanding result means that the expression system spontaneously released the enzyme directly in the biogenic vesicle delivery platform. Of note, both lysosomes and some EVs stem from the multivesicular endosomes (MVE), which may explain the high concentration of GLA and SGSH enzyme within the EVs. *In vitro* data demonstrate that GLA-loaded EVs were rapidly internalized and reached the lysosome sub-cellular target in cellular models of Fabry disease, inducing a therapeutic effect superior to the clinically used recombinant enzyme. The biodistribution of EV-GLA labeled with florescent DiR dye was investigated in a Fabry KO mice. EV-GLA were able to cross the BBB when administered intraarterially (i.a.) by canulation of the external carotid or intravenously (i.v.) by tail vein injection [Figure 1A and B]. *Ex vivo* images obtained by confocal microscopy indicated that the fluorescent signal of EV-GLA reached brain parenchyma after i.a. administration and, to a smaller extent, after i.v. administration [Figure 1C]. The enzymatic activity of GLA was measured in brain tissues 1 h post i.v. administration. Treated animals exhibited basal enzymatic activities, comparable to the K.O. animals [Figure 1D]. However, there are some sensitivity limitations regarding the techniques for measuring GLA activity and brain biodistribution. Therefore, the authors also evaluated the levels of globotriaosylceramide (Gb3, the enzyme substrate) in mice one week after a single i.v. administration of GLA and EV-GLA (1 mg/kg of GLA, Figure 1E). Remarkably, the results indicate that EV-GLA induced a significant reduction in Gb3 deposits in contrast to free GLA. Overall, these results

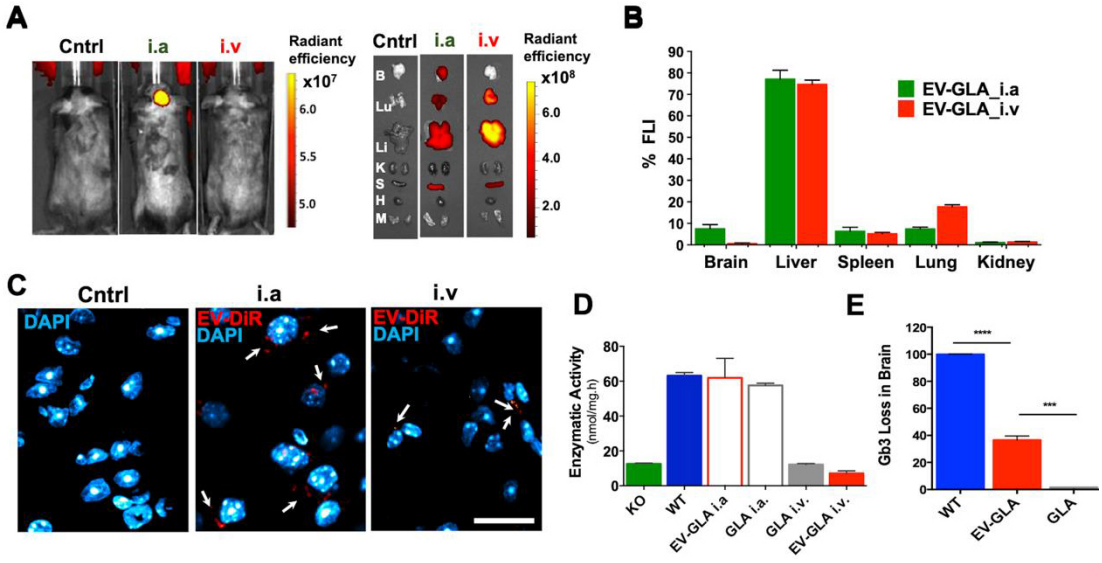

Figure 1. EV-GLA therapeutic effect. (A) *In vivo* (left) and *ex vivo* (right) fluorescence imaging (FLI) of Fabry KO mice treated with either intraarterial (i.a.) or intravenous (i.v.) administration of DiR-labeled EV-GLA (1 mg/kg of GLA) in comparison to the non-treated controls (Cntrl). (B) FLI signal quantification to compare the biodistribution of EV-GLA following i.v. or i.a. administration. (C) Confocal images of brain parenchyma showing DiR fluorescent signal of EV-GLA (red) and DAPI-labeled cell nuclei (blue). Scale bar represents 20 µm. (D) GLA enzymatic activity 1 h post-administration in brains of Fabry KO mice treated with GLA or EV-GLA via i.v. or i.a. administrations (E) Loss of Gb3 in KO mice that received an i.v. single dose of EV-GLA, as measured by LC-HRMS seven days after dosing (figure used with permission from Figure 5 of Seras-Franzoso *et al.* Available from: https://doi.org/10.1002/jev2.12058[25]). FLI: Luorescence imaging; GLA: alpha-galactosidase A.

indicate that i.v. administered EV-GLA reached the brain parenchyma and induced a therapeutic effect at lysosomes in Fabry KO mice.

Another proof-of-concept of EV efficacy in ERT therapy of LSDs was provided by Haney *et al.* in a preclinical model of Batten disease (BD)/neuronal ceroid lipofuscinosis (NCL)[31,32]. NCLs are a group of 14 inherited neurodegenerative disorders of childhood characterized by visual failure, epilepsy, progressive motor and cognitive decline, and premature death. NCLs are multisystemic disorders, but the brain is strongly affected with neurons progressively accumulating in lysosomes an NCL-specific lipopigment (lipofuscin) and some proteins such as the c-subunit of the mitochondrial ATP synthase (SCMA) before dying. In addition, an inflammatory response characterized by reactive astrocytosis is observed in the brain regions affected by the disease. There is currently no curative treatment available for most NCLs, except for type 2 (CLN2), which results from the defective activity of the soluble lysosomal hydrolase TPP1 (tripeptidyl peptidase-1). The approved ERT for CLN2, Brineura[33], significantly reduces motor and language decline in patients[34]. However, since the purified enzyme is delivered every two weeks via an intracerebroventricular device, some concerns exist about the long-term efficacy and tolerance of the treatment and alternative solutions are needed. The group of Elena Batrakova showed that macrophage-derived EVs loaded with purified TPP1 efficiently delivered the enzyme to lysosomes in both *in vitro* and *in vivo* models of CLN2. A robust accumulation of EV carriers was observed in the brain of a CLN2 mouse model after intraperitoneal injection[31]. Accumulation of storage material, astrocytosis, and neuronal death were reduced, and mouse lifespan was extended[31]. A further increase in CLN2 mouse model lifespan was obtained when EV-TPP1 was delivered by a combination of two delivery routes, intraperitoneal and intrathecal injections[32], which target both the brain and peripheral organs. Interestingly, intranasal administration of EV-TPP1 resulted in significant astrocytosis reduction and neuron survival increase[32]. This result gives some hope that EV-based therapy could cure brain consequences of CLN2 loss when delivered via a minimally invasive

administration route, an essential point for a lifelong treatment that would start during early childhood.

These encouraging results open a new venue in the field of ERT. Huge prospects may be expected considering that EVs are a versatile drug delivery platform endowed with intrinsic therapeutic properties and enhanced capabilities to cross biological barriers. From a market strategy standpoint, ERT may represent an attractive business niche for EV biotech companies considering treatment costs of ~€200,000 per patient-year. Additionally, the choice of an orphan indication is an asset to obtaining fast feedback from regulatory bodies. This would, to some extent, facilitate the entry of EV-based medicines in clinical trials with fewer patients, high reimbursement, and extended protection.

DECLARATIONS

Authors' contributions

Wrote the paper: Silva AKA, Sagné C
Revised the paper providing remarks for its improvement: Gazeau F, Abasolo I

Availability of data and materials

Not applicable.

Financial support and sponsorship

The authors acknowledge the French Agence Nationale de la Recherche (ANR) for funding via the action "Grand Défi - Bioproduction" (ANR-21-F2II-0001). Abasolo I was also granted by Instituto de Salud Carlos iii (grants PI18/00871 and PI21/00936, co-founded by European Regional Development Fund).

Conflicts of interest

Gazeau F and Silva AKA are co-founders of the spin-off Evora Biosciences. Silva AKA is co-founder of the spin-off EverZom. The other authors have no conflicts to declare.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Platt FM. Emptying the stores: lysosomal diseases and therapeutic strategies. *Nat Rev Drug Discov* 2018;17:133-50. DOI PubMed
2. Pandey MK, Burrow TA, Rani R, et al. Complement drives glucosylceramide accumulation and tissue inflammation in Gaucher disease. *Nature* 2017;543:108-12. DOI PubMed
3. Wraith JE. Lysosomal disorders. *Semin Neonatol* 2002;7:75-83. DOI PubMed
4. Solomon M, Muro S. Lysosomal enzyme replacement therapies: historical development, clinical outcomes, and future perspectives. *Adv Drug Deliv Rev* 2017;118:109-34. DOI PubMed PMC
5. Concolino D, Deodato F, Parini R. Enzyme replacement therapy: efficacy and limitations. *Ital J Pediatr* 2018;44:120. DOI PubMed PMC
6. Turner CT, Hopwood JJ, Brooks DA. Enzyme replacement therapy in mucopolysaccharidosis I: altered distribution and targeting of alpha-L-iduronidase in immunized rats. *Mol Genet Metab* 2000;69:277-85. DOI PubMed
7. Spada M, Pagliardini S, Yasuda M, et al. High incidence of later-onset fabry disease revealed by newborn screening. *Am J Hum Genet* 2006;79:31-40. DOI PubMed PMC
8. Hsu TR, Niu DM. Fabry disease: Review and experience during newborn screening. *Trends Cardiovasc Med* 2018;28:274-81. DOI

PubMed

9. Rombach SM, Hollak CE, Linthorst GE, Dijkgraaf MG. Cost-effectiveness of enzyme replacement therapy for Fabry disease. *Orphanet J Rare Dis* 2013;8:29. DOI PubMed PMC
10. Germain DP, Elliott PM, Falissard B, et al. The effect of enzyme replacement therapy on clinical outcomes in male patients with Fabry disease: a systematic literature review by a European panel of experts. *Mol Genet Metab Rep* 2019;19:100454. DOI PubMed PMC
11. Kishnani PS, Dickson PI, Muldowney L, et al. Immune response to enzyme replacement therapies in lysosomal storage diseases and the role of immune tolerance induction. *Mol Genet Metab* 2016;117:66-83. DOI PubMed
12. Le SQ, Kan SH, Clarke D et al. A humoral immune response alters the distribution of enzyme replacement therapy in murine mucopolysaccharidosis type I. *Mol Ther Methods Clin Dev* 2018;8:42-51. DOI PubMed PMC
13. Neuwelt E, Abbott NJ, Abrey L, et al. Strategies to advance translational research into brain barriers. *Lancet Neurol* 2008;7:84-96. DOI PubMed
14. Pardridge WM. Biopharmaceutical drug targeting to the brain. *J Drug Target* 2010;18:157-67. DOI PubMed
15. Pardridge WM. Blood-brain barrier delivery for lysosomal storage disorders with IgG-lysosomal enzyme fusion proteins. *Adv Drug Deliv Rev* 2022;184:114234. DOI PubMed
16. Zhu Y, Li X, Schuchman EH, Desnick RJ, Cheng SH. Dexamethasone-mediated up-regulation of the mannose receptor improves the delivery of recombinant glucocerebrosidase to Gaucher macrophages. *J Pharmacol Exp Ther* 2004;308:705-11. DOI PubMed
17. Safary A, Akbarzadeh Khiavi M, Mousavi R, Barar J, Rafi MA. Enzyme replacement therapies: what is the best option? *Bioimpacts* 2018;8:153-7. DOI PubMed PMC
18. Del Grosso A, Galliani M, Angella L, et al. Brain-targeted enzyme-loaded nanoparticles: A breach through the blood-brain barrier for enzyme replacement therapy in Krabbe disease. *Sci Adv* 2019;5:eaax7462. DOI PubMed PMC
19. Santi M, Finamore F, Cecchettini A, et al. Protein delivery by peptide-based stealth liposomes: a biomolecular insight into enzyme replacement therapy. *Mol Pharm* 2020;17:4510-21. DOI PubMed
20. Abasolo I, Seras-Franzoso J, Moltó-Abad M, et al. Nanotechnology-based approaches for treating lysosomal storage disorders, a focus on Fabry disease. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2021;13:e1684. DOI PubMed
21. Cabrera I, Abasolo I, Corchero JL, et al. α-Galactosidase-a loaded-nanoliposomes with enhanced enzymatic activity and intracellular penetration. *Adv Healthc Mater* 2016;5:829-40. DOI PubMed
22. Ansari NH, He Q, Cook JD, Wen J, Srivastava SK. Delivery of liposome-sequestered hydrophobic proteins to lysosomes of normal and batten disease cells. *J Neurosci Res* 1997;47:341-7. DOI PubMed
23. Choi H, Choi K, Choi C. Exosome-mediated delivery of active glucocerebrosidase to Gaucher model cells. *Molecular Genetics and Metabolism* 2019;126:S38-9. DOI
24. Do MA, Levy D, Brown A, Marriott G, Lu B. Targeted delivery of lysosomal enzymes to the endocytic compartment in human cells using engineered extracellular vesicles. *Sci Rep* 2019;9:17274. DOI PubMed PMC
25. Hugel B, Martínez MC, Kunzelmann C, Freyssinet JM. Membrane microparticles: two sides of the coin. *Physiology (Bethesda)* 2005;20:22-7. DOI PubMed
26. Ratajczak J, Wysoczynski M, Hayek F, Janowska-Wieczorek A, Ratajczak MZ. Membrane-derived microvesicles: important and underappreciated mediators of cell-to-cell communication. *Leukemia* 2006;20:1487-95. DOI PubMed
27. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. *Kidney Int* 2010;78:838-48. DOI PubMed
28. Matsumoto J, Stewart T, Banks WA, Zhang J. The transport mechanism of extracellular vesicles at the blood-brain barrier. *Curr Pharm Des* 2017;23:6206-14. DOI PubMed
29. Saint-Pol J, Gosselet F, Duban-Deweer S, Pottiez G, Karamanos Y. Targeting and crossing the blood-brain barrier with extracellular vesicles. *Cells* 2020;9:851. DOI PubMed PMC
30. Seras-Franzoso J, Díaz-Riascos ZV, Corchero JL, et al. Extracellular vesicles from recombinant cell factories improve the activity and efficacy of enzymes defective in lysosomal storage disorders. *J Extracell Vesicles* 2021;10:e12058. DOI PubMed PMC
31. Haney MJ, Klyachko NL, Harrison EB, Zhao Y, Kabanov AV, Batrakova EV. TPP1 Delivery To Lysosomes With Extracellular Vesicles And Their Enhanced Brain Distribution In The Animal Model Of Batten Disease. *Adv Healthc Mater* 2019;8:e1801271. DOI PubMed PMC
32. Haney MJ, Zhao Y, Jin YS, Batrakova EV. Extracellular vesicles as drug carriers for enzyme replacement therapy to treat CLN_2 batten disease: optimization of drug administration routes. *Cells* 2020;9:1273. DOI PubMed PMC
33. European Medicines Agency, Sciences Medicines. Health.Brineura cedipose alpha.
34. Schulz A, Ajayi T, Specchio N, et al; CLN2 Study Group. Study of intraventricular cerliponase Alfa for CLN_2 Disease. *N Engl J Med* 2018;378:1898-907. DOI PubMed