



New directions in the treatment of Gaucher disease

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Gaucher disease, an inherited metabolic disorder caused by mutations in the gene encoding acid- β -glucosidase (GlcCerase), is a multi-system disease whose manifestations include anemia, thrombocytopenia, hepatosplenomegaly, bone pathology and, in some cases, neurological signs. Enzyme replacement therapy (ERT) using recombinant GlcCerase (Cerezyme[®]) alleviates many disease symptoms and is used by ~3000 patients worldwide, and substrate-reduction therapy (SRT) using the glycolipid synthesis inhibitor *N*-butyldeoxynojirimycin [NB-DNJ (Zavesca[®])] has been approved recently for patients for whom ERT is unsuitable. It is our opinion that a multiplicity of treatment strategies is required for the management of Gaucher disease. In this article, we discuss the pros and cons of currently available treatments, and suggest complementary therapies arising from the determination of the X-ray structure of Cerezyme[®] and from delineation of secondary biochemical pathways affected in Gaucher disease.

Since the first description of Gaucher disease in 1882 by the French medical student Philippe Gaucher, an impressive body of clinical, biochemical, genetic and molecular work [1] has made Gaucher disease one of the best-studied inherited metabolic disorders [2]. Gaucher disease is a lysosomal storage disorder (LSD) caused by mutations in the gene encoding acid- β -glucosidase (GlcCerase), the enzyme that catalyzes the breakdown of the glycolipid glucosylceramide (GlcCer). These mutations result in GlcCer accumulation mainly in macrophages but also in other tissues. In rare cases, Gaucher disease is caused by mutations in the saposin C domain of the gene prosaposin [3], which encodes the saposin C activator protein that is required for optimal GlcCerase activity [4]. The disease occurs in three clinical forms. Type 1, the mildest and most common form, is characterized by varying degrees of hepatosplenomegaly (i.e. enlarged liver and spleen), anemia, thrombocytopenia, bone pains and skeletal lesions. Types 2 and 3 are both rare, with acute and fulminating (type 2), or heterogeneous and attenuated (type 3), neurological involvement accompanying visceral

manifestations. Type 1 Gaucher disease occurs with a frequency of 1 in 40 000–60 000 in the general population, and 1 in 500–1000 among Ashkenazi Jews [3].

Currently available treatments for Gaucher disease include enzyme replacement therapy (ERT) using Cerezyme[®] (Genzyme; <http://www.genzyme.com>), a recombinant form of GlcCerase [5], and substrate reduction therapy (SRT) using *N*-butyldeoxynojirimycin (NB-DNJ; Zavesca[®]) (Actelion; <http://www.actelion.com/Apps/WebObjects/Actelion>) [6]. The goal of both treatments is to reduce GlcCer storage, thus diminishing the deleterious effects caused by its accumulation. ERT achieves this by supplementing defective enzyme with active enzyme [7], whereas SRT works by lowering the rates of synthesis of all GlcCer-based glycolipids, thus reducing glycolipid accumulation. ERT has proved to be safe and effective over a period of >12 years, and a reduction in organ volumes, improvement in hematological parameters and amelioration of bone pains have dramatically improved the quality of life for many patients [5]. The pivotal clinical trial of Zavesca[®] was a non-comparative Phase I/II study in adult patients with mild-to-moderate type 1 disease who were unable or unwilling to receive ERT. Reductions in liver and spleen volumes were observed, although hematological responses were less impressive than those of patients who received ERT [8,9]. Other clinical trials have been, or are being performed, with Zavesca[®] [9,10], and a position statement on its use in treating type 1 Gaucher disease was published recently [11].

Are the currently available therapies sufficient?

Despite the impressive success of Cerezyme[®], and the recent availability of SRT, several issues remain unresolved concerning both treatments, consequently limiting their effectiveness and imposing burdens on patients. Among these are the dependence of ERT on intravenous infusions usually every 2 weeks, poor delivery of Cerezyme[®] to bones and lungs, and its inability to cross the blood–brain barrier (Table 1). By contrast, Zavesca[®] is given orally and crosses the blood–brain barrier [12], but causes several side-effects such as diarrhoea (in almost all patients during the first weeks of treatment), abdominal pains, tremor (in ~30% of patients) and peripheral neuropathy

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Table 1. The pros and cons of enzyme replacement therapy (ERT) and substrate reduction therapy (SRT)

Pros	Cons
ERT using Cerezyme[®] Safe and effective, with > 12 years experience in > 3000 patients worldwide Specifically reduces glucosylceramide storage	Requires life-long, intravenous infusions at least once every 2 weeks
Significantly improves many clinical symptoms, such as liver and spleen volumes, anemia and thrombocytopenia, bone pains and bone structure Improves general quality of life	Does not cross the blood–brain barrier and therefore cannot be used for neuronopathic Gaucher disease Poor effect on bones and lungs in patients with severe pre-existing lesions
SRT using Zavesca[®] Administered orally and therefore more convenient than ERT, with no intravenous-related complications	Expensive and therefore unavailable to patients in poor countries
Reduces glucosylceramide storage	Prevalence of short- and long-term adverse effects such as diarrhea, abdominal pains, loss of weight, tremor and peripheral neuropathy; therefore, currently only indicated to patients for whom ERT is unsuitable Efficacy not as dramatic as ERT, particularly in hematological parameters, and therefore suggested for patients with mild-to-moderate, but not severe, disease
Reduces organomegaly and causes improvement in hematological and biochemical parameters Crosses the blood–brain barrier and thus could help neuronopathic Gaucher disease patients An additional therapeutic option to ERT Non-antigenic	Concerns about potential effects on cognitive function
Proof of concept exists for application to other lysosomal storage disorders	Depletes all glucosylceramide-based glycolipids, which could cause additional adverse effects Expensive, and therefore also not an option in poor countries Contra-indicated in children and adolescents, and during pregnancy and lactation Adverse effects on spermatogenesis, and therefore male patients must stop the drug three months prior to planning a family Current formulation (Zavesca [®]) given 3 times a day, and requires good compliance

(in >10% of patients) (Table 1). Moreover, long-term reduction in glycolipid levels could affect a variety of cell functions because of the essential roles that these lipids play in normal cell physiology, which are becoming more apparent as glycolipid research diverges into new and unexpected areas [13,14]. Because of these problems, Zavesca[®] has been approved in Europe (including Israel) and the USA only for patients for whom ERT is ‘unsuitable’ and ‘not a therapeutic option’, respectively. Furthermore, patients who receive Zavesca[®] must undergo baseline and follow-up neurological and neuropsychological monitoring. Thus, we believe that significant improvements could be made in both ERT and SRT, and that new therapeutic approaches should be actively sought.

One example of a novel therapeutic approach for LSDs was reviewed recently in *TiPS* [15]. In this approach active-site-directed inhibitors are used both to stabilize the structure of the GlcCeramide enzyme and to serve as ‘pharmacological’ chaperones. Because some Gaucher disease mutations result in improperly folded GlcCeramide that is retarded in the endoplasmic reticulum and degraded there, such ‘chaperones’ might enhance normal trafficking of the enzyme through the secretory pathway, and thus increase its level in lysosomes. Proof of principle was obtained by incubating cultured cells expressing a mutant GlcCeramide (N370S) with sub-optimal concentrations of a GlcCeramide inhibitor, *N*-nonyl-deoxyjirimycin, which resulted in elevated enzyme activity [16]. A substantial amount of work is required before this approach could provide a therapeutic option for Gaucher disease and other LSDs (e.g. optimization of inhibitor levels in animal studies rather than in cultured cells, and determination of inhibitor efficacy in reducing GlcCer storage in the primary cell types and tissues affected in

Gaucher disease). However, such reservations are valid for any potential new treatment. We now argue that exploring ideas such as this, and exploring other novel approaches, will be of long-term benefit to Gaucher disease patients. Moreover, because of the relative prevalence of Gaucher disease among the LSDs, the lessons learned from its management and treatment should be of benefit to other LSDs. With this in mind, we now suggest some additional potential alternative and modified therapies, based both on the recent determination of the three-dimensional structure of Cerezyme[®] [17] and on studies aimed at delineating downstream biochemical pathways affected in Gaucher disease.

Suggestions for improved or complementary Gaucher disease therapies

The first approach is based on the three-dimensional structure of Cerezyme[®], which was recently solved to 2 Å resolution [17]. The structure comprises three non-contiguous domains that could not be predicted from the primary amino acid sequence (Figure 1). Domain I consists of one major three-stranded, anti-parallel β -sheet flanked by a perpendicular N-terminal strand and loop. Domain II consists of two closely associated β -sheets forming an independent domain resembling an immunoglobulin (Ig) fold. Domain III is a $(\beta/\alpha)_8$ (TIM) barrel containing the catalytic site. The function of the two non-catalytic domains is unknown but the location of mutations throughout all three domains suggests that they play important regulatory roles. No clear correlation was immediately apparent between the spatial location of particular mutants and the severity of clinical symptoms. However, some mutations located close to the active site mainly cause severe disease [17], whereas L444P, which

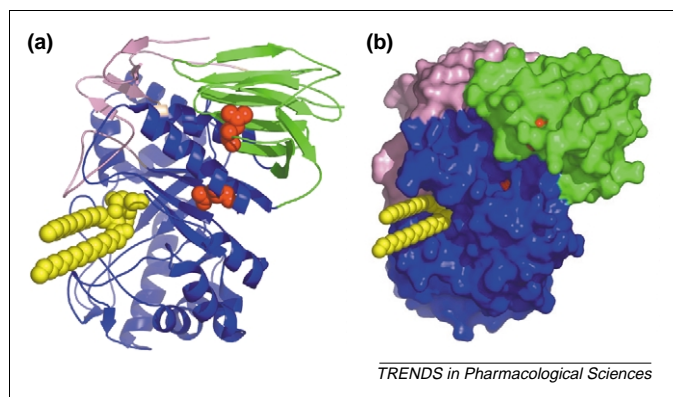


Figure 1. The three-dimensional structure of Cerezyme[®]. (a) A ribbon diagram, in which domain I is shown in purple, domain II is shown in green and domain III is shown in blue. Residues 370 (on domain III) and 444 (on domain II) are shown as red spheres, and the modeled glucosylceramide substrate on domain III is shown in yellow. (b) A surface plot is shown in the same orientation and with the same color coding.

always predisposes to severe disease (i.e. type 2 or 3) [2], is located on domain II, distant from the active site (Figure 1). Earlier studies suggested that L444P produces unstable protein [18], suggesting that the correct folding of domain II is required for optimal protein stability.

How does the availability of the Cerezyme[®] structure assist the development of alternative or improved therapies (Figure 2)? First, the tools of structure-based drug design should permit the design of activators to enhance the activity of either mutant enzyme(s), such as the common N370S mutation (Figure 1), which accounts for 70% of mutant alleles in Ashkenazi Jews and 25% of mutant alleles in non-Jews, or enzyme administered in ERT to enhance its stability and/or activity. Because approximately two-thirds of patients homozygous for N370S are asymptomatic and thus presumably have sufficient residual enzyme activity so that they do not develop symptoms, only a small degree of enzyme

activation would be necessary to provide enzyme with sufficiently enhanced levels of catalytic activity to overcome the effects of N370S, and possibly other mutations. Design of activators might require structural comparison of native GlcCeramide with mutant enzymes or with enzyme co-crystallized with small molecules; this should now be possible because crystallization conditions are established for Cerezyme[®] [17]. The second approach, and perhaps the most taxing, is engineering more-stable or more-active GlcCeramide. Although few studies have been published that systematically examine the fate of GlcCeramide after infusion (the main study was performed with Ceredase[®] [19], a first-generation, placental GlcCeramide), it is rapidly cleared from blood (within a few minutes) and has a half-life in the bone marrow of ~14 h [2]. Engineering a more-stable enzyme, or an enzyme with a higher catalytic activity, could reduce the number of infusions and potentially also reduce cost. Site-directed mutagenesis and directed evolution are the main tools in this approach, but the major challenge is determining which parts of the protein to alter. Substituting amino acids near or around the activity site could dramatically affect enzyme activity, and mutations of amino acids, or deletion and/or modification of structural determinants in the regulatory domains, such as insertion of suitably positioned disulfide bridges, could influence stability. In this regard, one important open question concerns the identification of the molecular mechanism by which saposin C enhances GlcCeramide activity. Resolving this issue will require either co-crystallization of saposin C with GlcCeramide, or rationale design of mutants, presumably in the non-catalytic domains that interfere with the ability of saposin C to modify GlcCeramide activity and/or binding to lysosomal membranes. Finally, the availability of the Cerezyme[®] structure permits a rationale approach to the production of small molecules for use as pharmacological chaperones (see above and

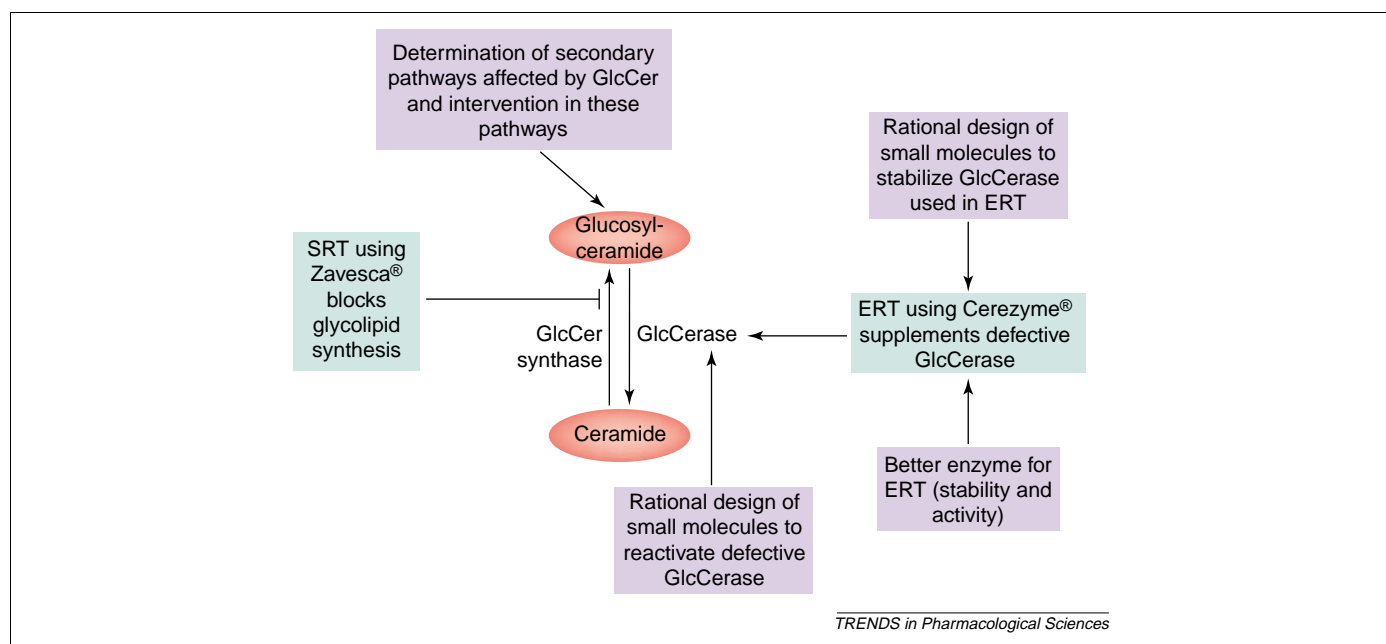


Figure 2. Potential new therapies for Gaucher disease. Glucosylceramide (GlyCer) is formed from ceramide by the enzyme glucosylceramide synthase, and degraded to ceramide by the enzyme glucosylceramidase (GlcCerase; acid- β -glucosidase). Current treatments are in shown green boxes, and potential new or modified treatments are shown in purple boxes. Abbreviations: ERT, enzyme replacement therapy; SRT, substrate reduction therapy.

Ref. [15]). In summary, the three-dimensional structure of Cerezyme[®] should pave the way for an exciting new era in ERT involving the use of small molecules or modified enzyme to increase ERT efficacy. Because clinical trials are underway for ERT in other LSDs, such as Fabry disease, Hurler syndrome and Pompe disease [7], lessons learned from attempts to create second-generation ERT for Gaucher disease should be of general benefit for other LSDs.

A second general approach to improved Gaucher disease therapy is based on the delineation of downstream pathways affected secondarily to GlcCer accumulation (Figure 2). Surprisingly, little is known about the molecular pathogenesis of Gaucher disease and a lack of basic research, perhaps as a result of the success of ERT, has hampered the development of new ideas and concepts during the past decade. For example, the size of the spleen increases up to 25-fold in patients with Gaucher disease but GlcCer in affected macrophages accounts for <2% of the additional tissue mass [20]. This implies that inflammatory responses might be involved [20], although evidence for the involvement of pro-inflammatory cytokines is relatively scant [1,2]. In addition, Gaucher cells are known to secrete various important mediators, such as cysteine proteases [21] and chemokines [22], that might also be involved in pathology. Other secondary pathways are also likely to be affected, and recent studies showing changes in phospholipid metabolism in neurons [23] and macrophages (A.H. Futerman *et al.*, unpublished), and changes in Ca²⁺ homeostasis [24,25] in models of Gaucher disease could provide unexpected clues about pathophysiological mechanisms. The involvement of secondary pathways is almost certain because sphingolipids and glycolipids, including GlcCer, are now recognized as important first and second messengers that regulate many cellular processes [26]. In summary, we suggest that determining the mechanistic relationship between the secondary metabolic pathways that are altered following GlcCer accumulation and cellular pathology is not only essential for a basic understanding of the disease mechanism, but also for developing new therapies and drugs. Such therapies could be used in conjunction with ERT or SRT, or alone in patients displaying relatively mild symptoms. Moreover, should a better enzyme become available for ERT, combinations of various treatments would provide physicians with a greater repertoire of therapeutic options than is currently available.

What is the future for Gaucher disease therapy?

Despite the success of Cerezyme[®] in ERT, we believe that significant progress in Gaucher disease therapy could be made by improving ERT, optimizing SRT and developing new therapies. Importantly, although the number of Gaucher disease patients is relatively small, this disease imposes a disproportionate burden on the healthcare budget of several countries [27], particularly those with limited resources. In this regard, there is only one commercial source of GlcCer for ERT, and competition, such as that which exists for ERT for Fabry disease [in which two companies, Genzyme and Transkaryotic Therapies (<http://www.tktx.com/patient/products.htm>),

are producing recombinant α -galactosidase A], would presumably drive down cost. Moreover, lessons learned from studies of Gaucher disease management will be applicable to other LSDs for which either ERT, SRT or small-drug therapies are being developed. Irrespective of commercial and economic considerations, we believe that the time is ripe to expand the horizons of Gaucher disease therapy and of basic research, and eventually apply lessons learned from the study of this fascinating multi-systemic disease to other LSDs, and to other metabolic diseases involving defective sphingolipid or glycolipid metabolism.

References

- Zimran, A. ed. (1997) *Gaucher's Disease* Balliere Tindall
- Beutler, E. and Grabowski, G.A. (2001) Gaucher disease. In *The Metabolic and Molecular Bases of Inherited Disease* (Vol. II) (Scriver, C.R. *et al.*, eds), pp. 3635–3668, McGraw-Hill
- Horowitz, M. and Zimran, A. (1994) Mutations causing gaucher disease. *Hum. Mutat.* 3, 1–11
- Zhao, H. and Grabowski, G.A. (2002) Gaucher disease: perspectives on a prototype lysosomal disease. *Cell. Mol. Life Sci.* 59, 694–707
- Weinreb, N.J. *et al.* (2002) Effectiveness of enzyme replacement therapy in 1028 patients with type 1 Gaucher disease after 2 to 5 years of treatment: a report from the Gaucher registry. *Am. J. Med.* 113, 112–119
- Lachmann, R.H. (2003) Miglustat. Oxford GlycoSciences/Actelion. *Curr. Opin. Investig. Drugs* 4, 472–479
- Grabowski, G.A. and Hopkin, R.J. (2003) Enzyme therapy for lysosomal storage disease: principles, practice, and prospects. *Annu. Rev. Genomics Hum. Genet.* 4, 403–436
- Cox, T. *et al.* (2000) Novel oral treatment of Gaucher's disease with N-butyldeoxynojirimycin (OGT 918) to decrease substrate biosynthesis. *Lancet* 355, 1481–1485
- Zimran, A. and Elstein, D. (2003) Gaucher disease and the clinical experience with substrate reduction therapy. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358, 961–966
- Heitner, R. *et al.* (2002) Low-dose N-butyldeoxynojirimycin (OGT 918) for type I Gaucher disease. *Blood Cells Mol. Dis.* 28, 127–133
- Cox, T.M. *et al.* (2003) The role of the iminosugar N-butyldeoxynojirimycin (miglustat) in the management of type 1 (non-neuronopathic) Gaucher disease: a position statement. *J. Inherit. Metab. Dis.* 26, 513–526
- Platt, F.M. *et al.* (1997) Prevention of lysosomal storage disease in Tay-Sachs mice treated with N-butyldeoxynojirimycin. *Science* 276, 428–431
- Kolter, T. *et al.* (2002) Combinatorial ganglioside biosynthesis. *J. Biol. Chem.* 277, 25859–25862
- Buccoliero, R. *et al.* (2002) The role of sphingolipids in neuronal development: lessons from models of sphingolipid storage diseases. *Neurochem. Res.* 27, 565–574
- Fan, J.Q. (2003) A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. *Trends Pharmacol. Sci.* 24, 355–360
- Sawkar, A.R. *et al.* (2002) Chemical chaperones increase the cellular activity of N370S β -glucosidase: a therapeutic strategy for Gaucher disease. *Proc. Natl. Acad. Sci. U. S. A.* 99, 15428–15433
- Dvir, H. *et al.* (2003) X-ray structure of human acid-beta-glucosidase, the defective enzyme in Gaucher disease. *EMBO Rep.* 4, 704–709
- Grace, M.E. *et al.* (1994) Analysis of human acid β -glucosidase by site-directed mutagenesis and heterologous expression. *J. Biol. Chem.* 269, 2283–2291
- Mistry, P.K. *et al.* (1996) Therapeutic delivery of proteins to macrophages: implications for treatment of Gaucher's disease. *Lancet* 348, 1555–1559
- Cox, T.M. (2001) Gaucher disease: understanding the molecular pathogenesis of sphingolipidoses. *J. Inherit. Metab. Dis.* 24 (Suppl. 2), 106–121
- Moran, M.T. *et al.* (2000) Pathologic gene expression in Gaucher

- disease: up-regulation of cysteine proteinases including osteoclastic cathepsin K. *Blood* 96, 1969–1978
- 22 Boot, R.G. *et al.* (2004) Marked elevation of the chemokine CCL18/PARC in Gaucher disease: a novel surrogate marker for assessing therapeutic intervention. *Blood* 103, 33–39
- 23 Bodennec, J. *et al.* (2002) Phosphatidylcholine synthesis is elevated in neuronal models of Gaucher disease due to direct activation of CTP:phosphocholine cytidyltransferase by glucosylceramide. *FASEB J.* 16, 1814–1816
- 24 Korkotian, E. *et al.* (1999) Elevation of intracellular glucosylceramide levels results in an increase in endoplasmic reticulum density and in functional calcium stores in cultured neurons. *J. Biol. Chem.* 274, 21673–21678
- 25 Lloyd-Evans, E. *et al.* (2003) Glucosylceramide and glucosylsphingosine modulate calcium mobilization from brain microsomes via different mechanisms. *J. Biol. Chem.* 278, 23594–23599
- 26 Smith, W.L. and Merrill, A.H. Jr (2002) Sphingolipid metabolism and signaling minireview series. *J. Biol. Chem.* 277, 25841–25842
- 27 Beutler, E. (1994) Economic malpractice in the treatment of Gaucher's disease. *Am. J. Med.* 97, 1–2

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