Minireview

Taliglucerase alfa: An enzyme replacement therapy using plant cell expression technology

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Abstract

Gaucher disease (GD) is a rare, genetic lysosomal storage disorder caused by functional defects of acid β-glucosidase that results in multiple organ dysfunction. Glycosylation of recombinant acid human β-glucosidase and exposure of terminal mannose residues are critical to the success of enzyme replacement therapy (ERT) for the treatment of visceral and hematologic manifestations in GD. Three commercially available ERT products for treatment of GD type 1 (GD1) include imiglucerase, velaglucerase alfa, and taliglucerase alfa. Imiglucerase and velaglucerase alfa are produced in different mammalian cell systems and require production glycosylation modifications to expose terminal α-mannose residues, which are needed for mannose receptor-mediated uptake by target macrophages. Such modifications add to production costs. Taliglucerase alfa is a plant cell-expressed acid β-glucosidase approved in the United States and other countries for ERT in adults with GD1. A plant-based expression system, using carrot root cell cultures, was developed for production of taliglucerase alfa and does not require additional processing for postproduction glycosidic modifications. Clinical trials have demonstrated that taliglucerase alfa is efficacious, with a well-established safety profile in adult, ERT-naïve patients with symptomatic GD1, and for such patients previously treated with imiglucerase. These included significant improvements in organomegaly and hematologic parameters as early as 6 months, and maintenance of achieved therapeutic values in previously treated patients. Ongoing clinical trials will further characterize the long-term efficacy and safety of taliglucerase alfa in more diverse patient populations, and may help to guide clinical decisions for achieving optimal outcomes for patients with GD1.

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1. Introduction

Gaucher disease (GD) is a rare lysosomal storage disorder that results from mutations in the gene (GBA1) encoding acid β-glucosidase (glucocerebrosidase [EC 3.2.1.45]) [1]. Insufficient enzyme activity leads to accumulation of glucocerebrosidase and other glucosphingolipids within the lysosomes of various cells and tissues, which results in varying degrees of visceral, bone, and neuronal pathology [1]. Three types of GD have been identified; GD type 1 (GD1) is the most common in the Western world, with clinical manifestations of hepatosplenomegaly, anemia, thrombocytopenia, and bone and bone marrow disease, but a lack of early onset primary central nervous system involvement. The
latter is characteristic of GD types 2 and 3. Currently, enzyme replacement therapy (ERT) with repeated intravenous infusions of recombinant, active acid β-glucosidase ameliorates many of the visceral signs and symptoms, and is recommended for patients significantly affected by GD1 [1,2].

Early attempts to treat GD1 with intravenous ERT were unsuccessful and provided limited therapeutic benefit, primarily because: 1) exogenous administration of unmodified human enzyme derived from placenta did not effectively enter target cells [3]; and 2) the amounts of such enzyme preparations were severely limited. Modern ERT evolved based on the ability to produce large amounts of human enzymes by recombinant methods and discovery of the macrophage mannose receptors (MMRs) for preferential uptake of mannose-terminated glycoproteins including acid β-glucosidase, by tissue macrophages [4,5]. This finding spurred development of more effective recombinant human acid β-glucosidase products, modified using various posttranslational or postproduction glycosylation methods to expose terminal α-mannose residues [6,7]. Currently, three ERTs are commercially available for the treatment of GD1; two are produced in mammalian cell-based systems. Imiglucerase (Genzyme, a Sanofi Corporation, Cambridge, MA, USA) is produced in a Chinese hamster ovary [CHO] system and velaglucerase alfa (Shire Pharmaceuticals Inc., Dublin, Ireland) is produced in a human fibrosarcoma cell line [8–10]. Taliglucerase alfa (Protalix Biotherapeutics, Carmiel, Israel) is a new acid β-glucosidase ERT that is approved by the US Food and Drug Administration for the treatment of GD1 in adults in the United States [11]; it is also approved in Israel and other countries, and is the first plant cell-expressed biotherapeutic approved for use in humans [12].

While the use of mammalian cell-based systems for the production of biotherapeutics is well established [13], manipulation of plant-based platforms for production of biotherapeutic agents is relatively new [14]. In recent years, researchers have successfully produced recombinant human proteins from whole-plant, transgenic crops [15]. In addition to transgenic plants, other production platforms are available, such as those based on plant-transient expression technology (virus-based or not), which are being used for vaccine antigen production [16]. Taliglucerase alfa is unique in that it is a plant cell-expressed biotherapeutic produced in a closed, sterile culture system [12]. Because of the novelty of the plant cell-based protein production system, a key purpose of this review is to describe the production technology for taliglucerase alfa. Furthermore, the clinical implications of this enzyme expression system will be discussed in the context of completed and ongoing clinical studies of this novel ERT.

2. Expression and production platforms for biotherapeutics

As the development and manipulation of expression platforms for biotherapeutics have progressed, distinct advantages and disadvantages have emerged with each approach (Table 1) [14,17–19]. Advantages of prokaryotic production systems (e.g., *Escherichia coli*) include ease of genetic manipulation, rapid cell growth, and high levels of protein expression [13]. However, prokaryotic systems are not ideal for the production of complex proteins that require posttranslational modifications for therapeutic efficacy. Posttranslational modifications of protein folding or disulﬁde bond formation are difﬁcult to achieve with prokaryotic systems, and posttranslational glycosylation processing is not possible.

Since yeast- and mammalian cell-based production systems are capable of providing posttranslational modifications, they provide for the production of therapeutically active proteins compared with prokaryotic platforms [13,20]. These systems can be more expensive than prokaryotic systems, specifically in terms of culture processes, operating costs, and scale-up difﬁculties [18]. Furthermore, compared with prokaryotic systems, these systems typically produce lower yields [13,18,19].

Plant-based production systems share some advantages and disadvantages of both prokaryotic and mammalian systems. Like prokaryotic systems, plant cell-based systems are associated with low culture costs, as they permit use of simple synthetic media, and allow rapid scale-up of the enzyme product [14,17–19]. In addition, plant-based systems are capable of expressing complex proteins, and effectively achieve posttranslational modifications, specifically N-glycosylation, as with other widely used yeast and mammalian systems [13,14,21]. Because plant cell-based systems do not use cells or culture media from animal sources, expression products are not susceptible to contamination with mammalian pathogens [14]. Moreover, plant cell cultures present a natural barrier to mammalian pathogen contamination, as attempts to propagate mammalian viruses in plant cells have been unsuccessful [22]. Nevertheless, plant-based systems present distinct challenges, depending on the specific plant system utilized [14,19].

2.1. Taliglucerase alfa plant-based production platform

Taliglucerase alfa is produced using the novel ProCellEx® plant cell-based protein expression system [12], in which plant cells are cultured in suspension in a closed bioreactor (Fig. 1). Isolated carrot root cells were stably transformed with *Agrobacterium tumefaciens* carrying the binary Ti-plasmid vector harboring the acid β-glucosidase CDNA and the kanamycin resistance gene (*NPTII*). Transformed cells were selected by antibiotic resistance, and clonal selection was performed by analysis of protein expression levels in transgenic calli grown on solid medium [14,23]. The selected clone was further expanded in suspension to develop a master cell bank consisting of frozen cell aliquots similar to the mammalian systems, in order to provide a continuous supply of fresh cells for inoculation into bioreactors [24].

For cell culture, the thawed cells were initially seeded in solid media, and the resulting cell mass was used to innoculate the liquid medium.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Overview of the advantages and disadvantages of protein expression systems.</th>
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<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td><strong>Disadvantages</strong></td>
</tr>
<tr>
<td>Prokaryotic systems [14,17,18]</td>
<td>Difficulty with posttranslational modifications (signal peptide cleavage, propeptide processing, protein folding, disulﬁde bond formation, and glycosylation)</td>
</tr>
<tr>
<td>• Ease of genetic manipulation</td>
<td></td>
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<tr>
<td>• Rapid growth</td>
<td>High costs</td>
</tr>
<tr>
<td>• High expression level</td>
<td>Low yields</td>
</tr>
<tr>
<td>Mammalian-based systems [14,17–19]</td>
<td>Scale-up difficulties</td>
</tr>
<tr>
<td>• Improved posttranslational modification</td>
<td></td>
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<tr>
<td>• Production of therapeutically active proteins</td>
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<tr>
<td>Plant-based systems [14,17]</td>
<td>Protein instability/degradation</td>
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<tr>
<td>• Lower costs</td>
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<tr>
<td>• Easy transformation</td>
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<tr>
<td>• Improved posttranslational modification</td>
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<tr>
<td>• Decreased risk of contamination with mammalian pathogens</td>
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<tr>
<td>• Efficient, readily scaled up</td>
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</table>
The ProCellEx-contained bioreactor system [12] uses aqueous media consisting of highly purified water and defined inorganic nutrients and plant growth factors to promote rapid growth and prevent differentiation. The media are contained within bioreactors which are disposable, recyclable, sterile, large, flexible plastic containers, in which cells are grown and harvested in consecutive cycles, with a central unit providing oxygen and nutrients. The bioreactors allow for the removal of excess air and waste gases, as well as the introduction of fresh inoculants and culture media. The disposable bioreactors are scaled-up horizontally, which allows for expanded production without changing growth conditions or affecting characteristics of the final product, and yields increased batch-to-batch reproducibility.

In other plant culture systems, the protein of interest is secreted into the culture medium to obtain mature type N-glycans. In comparison, the expressed protein in taliglucerase alfa is designed to be uniquely stored within the neutral storage vacuole of the carrot cells. In that compartment, the terminal glycosidic residues are removed, thereby exposing α-mannosyl residues. Furthermore, the localization of the expressed enzyme within the storage vacuoles guards against posttranslational degradation, as this organelle contains relatively low protease levels [23]. Analysis of N-glycosylation patterns demonstrated that the final taliglucerase alfa product exhibits a highly homogenous glycan profile with more than 90% tri-mannose structures [23,25]. Extraction of the plant cell-expressed acid β-glucosidase involves harvesting and detergent solubilization of the cultured cells followed by purification with cation exchange and hydrophobic chromatography [23].

2.1.1. The taliglucerase alfa construct

Production of taliglucerase alfa required codon usage optimization of the sequence of acid β-glucosidase to allow sufficient expression of the recombinant enzyme [23]. First, the cDNA encoding acid β-glucosidase was subcloned into a plasmid containing the signal peptide from Arabidopsis endochitinase to facilitate translocation into the endoplasmic reticulum. Two amino acids that originate from the linker used for fusion of the signal peptide remain at the N-terminus of the final protein product. The plasmid also contains nucleotides encoding for the C-terminal addition of the vacuolar targeting sequence from tobacco chitinase A; this facilitates the targeted storage of expressed protein within cellular vacuoles. Seven amino acids from this chitinase A sequence remain in the final protein product. Another change in the enzyme sequence includes a His-Arg substitution at position 495; this substitution is also present in imiglucerase [26]. None of these modifications are thought to substantially alter enzyme function in vitro or in vivo [23,27,28]. A comparative study of the in vitro and in vivo functionality of the three marketed enzymes, using the same analytical methodology, shows no functional differences between the enzymes [25].

3. Taliglucerase alfa structure and targeting

A GD1 ERT product should have normal catalytic activity and stability. In addition, the recombinant acid β-glucosidase needs to have appropriate posttranslational glycosylation that results in both high-mannose residues and complex oligosaccharide chains for appropriate, preferential targeting to the cells of pathology—the macrophages [29]. Specifically, for optimal macrophage receptor-mediated internalization of the protein, terminal α-mannose residues are needed on the glycan chains [3,29]; core or central localization of the mannose residues is associated with reduced enzyme uptake [3,30].

4. Uptake of exogenously administered taliglucerase alfa

Wild-type acid β-glucosidase (EC 3.2.1.45; glucosidase hydrolase A family) is encoded by GBA1 that maps to chromosome 1q21. In mammalian cells, following transcription, biosynthesis of the nascent protein (Fig. 2) [31,32] within the endoplasmic reticulum occurs over the course of about 3 h; the nascent polypeptide acquires, by en bloc transfer from mannosyl-dolichol-phosphate, a high-mannose oligosaccharide structure. The protein is then transported to the Golgi apparatus, where it is further modified and undergoes differential remodeling of its oligosaccharide chains. This involves removal of many mannosyl residues and the addition of N-acetylgalactosamine, galactose, and sialic acid in its cis- to trans-Golgi movement over 3 h [33] to yield a high molecular weight enzyme (Mr ~59,000–62,000) containing 497 amino acids [1].

Fig. 1. Diagram of taliglucerase alfa production. Photographs courtesy of Protalix Biotherapeutics, Carmiel, Israel.
Four of the five N-glycosylation sites are normally occupied in mature acid β-glucosidase, and co-translational glycosylation is required for the formation of a catalytically active conformer [34]. While still within the endoplasmic reticulum and Golgi, the maturing enzyme binds with lysosomal integral membrane protein-2 (LIMP-2), which initiates transfer from the Golgi to lysosomes via a mannose-6-phosphate receptor-independent mechanism [32]. Bound with LIMP-2, acid β-glucosidase travels to more distal portions of the cell, and is eventually taken up into dense lysosomes, where it dissociates from LIMP-2, and resides on the lysosomal luminal membrane [32]. Normally in mammalian cells, acid β-glucosidase is tightly associated with the inner lysosomal membrane and is not secreted in any significant amount.

The process of synthesis and oligosaccharyl remodeling is similar in mammalian and plant cells with some differences. The primary difference is the ability of plant cells to produce terminal α-mannosylated residues as a natural product of vacuole oligosaccharyl remodeling after initial endoplasmic reticulum and Golgi glycan attachment in proteins targeted to this compartment [18,35]. Taliglucerase alfa enzyme contains a targeting signal for the vacuole, chitinase A, and therefore is targeted to that organelle.

Compared with an intracellularly synthesized enzyme, uptake of intravenously administered recombinant acid β-glucosidase ERT uses a completely different receptor for trafficking to the lysosomes. This is mediated by MMRs that reside on the outer surface of the macrophage plasma membrane (Fig. 2) [31,32]. The terminal α-mannose residues on the recombinant enzymes bind with surface MMRs, the glycan/receptor complexes are endocytosed, and endosomes form within the macrophage that eventually fuse with lysosomes. Either in the late endosome or lysosome, the biologically active recombinant acid β-glucosidase gains access to and hydrolyzes the stored glucosylceramide in GD macrophages [31]. This targeting is preferential because the GDI agents can be delivered to other cellular types by yet unknown mechanisms [25,36]. However, because binding of glycan mannose residues to MMRs is essential in the macrophage uptake process [37], improper glycosylation may impede adequate uptake of infused ERT [1,26,31].

In line with the known capacity for plant-derived cell culture systems to produce complex proteins [18,35], production of taliglucerase alfa in the carrot cell culture system requires no additional steps or reagents to yield a mature enzyme with exposed terminal mannose structures [23]. The dominant glycan is the core structure found in most glycoproteins purified from other plant-based systems; the enzyme also contains core α-(1,2)-xylose and core α-(1,3)-fucose [23]. This is in contrast to mammalian glycans, which typically have core α-(1,6)-fucose and do not contain xylose [18].

In comparison, manufacture of imiglucerase from the acid β-glucosidase produced in CHO cells requires postproduction sequential deglycosylation with specific exoglycosidases, α-neuraminidase, β-galactosidase, and β-N-acetylglucosaminidase to expose terminal α-mannose residues [37,38]. X-ray crystallography studies of the endoF, partially deglycosylated, final imiglucerase product and the isolated glycans, show complex-type glycans exhibiting core fucosylation terminating with the N-acetylglucosamine tri-mannosyl core, and microheterogeneity at each glycosylation site. At sites N146 and N270, high-mannose-type glycans contain five to six mannose units with one mannose-6-phosphate [26]. Similarly, velaglucerase alfa, expressed in a human fibrosarcoma cell culture system, requires co-culture with kifunensine, an α-mannosidase I inhibitor, to yield secretion of the enzyme decorated with high-mannose glycans [39]. Detailed glycan map analysis of the velaglucerase alfa protein shows that it contains six to nine mannose units; glycosylation sites are heterogeneous and also contain mannose-6-phosphate [26]. Velaglucerase alfa also contains monosylated, monoantennary hybrid, and complex-type structures with core fucosylation [26].

In a recent head-to-head comparison of the glycosylation profiles of the three currently available recombinant enzymes [25], unique patterns of terminal residues and mannose chain length were observed. As discussed previously, the glycan profile of taliglucerase alfa was approximately 90% tri-mannose structures, with a β-(1,2)-xylose attached to the bisecting mannose and an α-(1,3)-fucose attached to

the reducing N-acetylgalcosamine (Fig. 3) [25]. The predominant glycans for imiglucerase consisted of pauci-mannose structures attached to a chitobiose tri-mannosyl core [26] with an addition of either a core α-(1,6)-linked fucose attached to the reducing N-acetylgalcosamine, or an additional terminal N-acetylgalcosamine (Fig. 3). The remaining glycans were of variable type, containing additional N-acetylgalcosamine, galactose, and sialic acid residues. The glyccan profile of velaglucerase alfa was approximately 90% high-mannose structures, with 9 mannoses attached to a chitobiose core being the predominant form (Fig. 3). Both taliglucerase alfa and velaglucerase alfa showed about 100% exposed mannose residues. Despite this structural heterogeneity, no significant differences were observed in the enzymatic activity, stability, and macrophage uptake capacity among the three recombinant enzymes [25]. However, a separate study by Brunsstein et al. [40] showed that velaglucerase alfa had a 2.5-fold faster rate of uptake into macrophages of U937 cells compared with imiglucerase. In addition, Berger et al. [41] reported a lower efficiency of uptake for taliglucerase alfa compared with imiglucerase and velaglucerase alfa in monocytes from patients with GD1. These discrepancies may be attributed to the different types of cells or culture conditions used in these studies.

Core β-(1,2)-xylose- and core α-(1,3)-fucose-containing glycoepitopes are considered important IgE binding determinants on plant allergens and have the potential to elicit IgG production in humans [35], which can lead to rapid clearance from serum [21]. In pre-clinical studies, rats and selected mouse strains developed weak antibodies to these core β-(1,2)-xylose- and α-(1,3)-fucose epitopes. One study established that 50% of sera from nonallergic human blood donors have antibody titers specific to core β-(1,2)-xylose and 25% for core α-(1,3)-fucose [42]. However, the clinical significance of these data remains speculative [18,42,45]. In other studies, a plant recombinant monoclonal murine IgG antibody produced in transgenic plants with plant-specific glycans was found to be non-immunogenic in mice [44]. In a murine model with Wolman disease using plant-derived lysosomal acid lipase, no evidence was found for antibodies to plant oligosaccharides and while antibodies to the protein were found, efficacy was unaffected [43].

5. Taliglucerase alfa clinical trials

Patients with GD1 have been enrolled in several clinical studies, as well as compassionate use programs, to receive treatment with taliglucerase alfa. Some of these clinical trials are currently under way or have recently been completed (Table 2) [45–49]. The studies were carried out in several centers worldwide and were designed to assess the efficacy and safety of taliglucerase alfa in ERT-naïve adult [49,50] and pediatric [48,51] patients, and in ERT-experienced adult and pediatric patients switched from imiglucerase to taliglucerase alfa [47,52].

Taliglucerase alfa 30 U/kg and 60 U/kg were efficacious in treatment-naive adults in study PB-06-001 [49] and in treatment-naive pediatric patients in study PB-06-005 [48,51]; these patients received biweekly treatment with taliglucerase alfa for 9 and 12 months, respectively. Taliglucerase alfa was well tolerated, and meaningful and sustained improvement of the disease state was observed in all clinically relevant parameters. Furthermore, in study PB-06-002, at the end of 9 months of treatment with taliglucerase alfa, mean organ volumes and hematologic values remained stable in treatment-experienced pediatric and adult patients who switched from imiglucerase to taliglucerase alfa [47,52]. Overall, the efficacy results in children were comparable with those in adults with no evidence of the need for dose adjustment in children. Results of extension study PB-06-003 [45] showed continued reduction in spleen and liver volumes, chitotriosidase activity, and improvement in hemoglobin levels and platelet counts following 36 months of taliglucerase alfa in treatment-naive adult patients [50] and 24 months of treatment with taliglucerase alfa in treatment-experienced adult and pediatric patients switched from imiglucerase to taliglucerase alfa [52].

For the completed studies, PB-06-001 [49], PB-06-002 [47], and PB-06-005 [48,51], safety data were collected from 74 patients aged 2 to 74 years (58 adult and 16 pediatric patients) who were exposed to taliglucerase alfa doses ranging from 11 to 69 U/kg every 2 weeks. A total of 47 patients (39 adult and 8 pediatric patients) were treated for ≥2 years and 23 patients were treated ≥3 years. Safety results were as expected for this biologic treatment. Overall, taliglucerase alfa was safe and well tolerated in the studied populations. The most clinically significant risk associated with treatment with taliglucerase alfa was hypersensitivity reactions. As with all biotherapeutic proteins, administration of taliglucerase alfa has resulted in formation of IgG anti-antibody antibodies in clinical studies of both adult and pediatric patients. No clear relationship was observed between the development of these antibodies and the emergence of adverse events. These data provide proof-of-principle for production of glycosylated recombinant human proteins in a non-mammalian, plant cell-derived expression system.

6. Conclusions

Within the last 20 years, a paradigm shift in the treatment of GD1 occurred with the successful development of ERT that targeted mannose-receptor-mediated macrophage uptake. Recently, physician readers of Nature Reviews Drug Discovery named the first ERT developed for patients with GD1 (alglucerase) as the most transformational drug in the field of genetics in the past 20 years [53,54]. This acknowledgment underscores the critical impact the development of subsequent ERTs, including taliglucerase alfa, has made in the lives of patients with GD1. Currently, three recombinant human β-glucosidase formulations are available: imiglucerase and velaglucerase alfa—produced in mammalian-based cell systems [8,9], and taliglucerase alfa—a novel, plant cell-expressed biotherapeutic [11,12]. Treatment with all of these formulations has proven safe and effective, marked by significant reductions in disease-related organomegaly and improvements in anaemia and other hematologic parameters [49,55,56]. Overall, adult patients with GD1 have improved health resulting from treatment with these ERTs, leaving no question that ERT has been an unqualified success for this rare disease [54].

Imiglucerase, velaglucerase alfa, and taliglucerase alfa are each produced in different expression systems and undergo various modifications to expose their mannose residues [23,37–39]. While these three ERTs have some differences in their glycosylation profile, including variation of terminal residues and mannose chain length [25], these differences do not significantly alter their in vitro or ex vivo properties. The stabilities, catalytic rate constants, and overall X-ray structures of the three ERTs are highly similar, if not identical [26]. Currently, no major differences can be discerned between these drugs in humans; their safety and efficacy profiles in patients with GD1 are similar.

It is important to note that in the context of enzyme replacement, it is possible to have MMR-independent targeting. In 2005, Du and colleagues [57] demonstrated that administration of purified, human lysosomal acid lipase (LAL), expressed in Pichia pastoris, improved hepatocytes and visceral macrophages in mice with no LAL or MMR. Specifically, the presence of high mannose residues on the LAL enzyme resulted in preferential, if not exclusive, uptake by hepatic cells, particularly Kupffer cells. These findings implicate MMR-independent pathways in the in vivo uptake and delivery of human LAL to lysosomes. A later study—the first to assess enzyme replacement via intraperitoneal injection with a human protein produced in a plant cell-based expression system—reported similar findings [43]. These observations highlight the importance of targeting glycoproteins via specific uptake mechanisms to achieve a therapeutic effect on lysosomal storage: the effects may be mediated preferentially by MMR, but other mechanisms may become preferential when MMR is missing, blocked, or down-regulated [57].
Table 2
Summary of phase III–IV clinical trials of taliglucerase alfa safety and efficacy.

<table>
<thead>
<tr>
<th>Study name</th>
<th>Trial identifier</th>
<th>Phase</th>
<th>Design</th>
<th>Patients</th>
<th>Taliglucerase alfa dosing</th>
<th>End points</th>
</tr>
</thead>
<tbody>
<tr>
<td>PB-06-001</td>
<td>NCT00376168</td>
<td>III</td>
<td>9-Month, double-blind, randomized, parallel-group, multicenter, dose-ranging, pivotal trial</td>
<td>Treatment-naive patients with GD</td>
<td>30 or 60 U/kg every 2 weeks</td>
<td>Change from baseline in spleen volumes, liver volumes, platelet counts, hemoglobin levels, and chitotriosidase activity</td>
</tr>
<tr>
<td>PB-06-002</td>
<td>NCT00712348</td>
<td>III</td>
<td>9-Month, open-label, switchover trial</td>
<td>Patients with stable GD treated with imiglucerase in previous 2 years</td>
<td>Dose equivalent to current imiglucerase every 2 weeks</td>
<td>Change from baseline in spleen volumes, liver volumes, platelet counts, hemoglobin levels, and chitotriosidase activity</td>
</tr>
<tr>
<td>PB-06-003</td>
<td>NCT00759939</td>
<td>III</td>
<td>15-Month, open-label extension trial</td>
<td>Patients with GD who successfully completed 9-month, phase III pivotal trial (PB-06-001)</td>
<td>30 or 60 U/kg every 2 weeks (total 20 infusions)</td>
<td>Change from baseline in spleen volumes, liver volumes, platelet counts, hemoglobin levels, and chitotriosidase activity</td>
</tr>
<tr>
<td>PB-06-004</td>
<td>NCT00962260</td>
<td>NA</td>
<td>Expanded access trial, open-label</td>
<td>Adult patients with non-neuronopathic disease, prior ERT with imiglucerase discontinued or reduced due to shortage</td>
<td>Dose every 2 weeks; dose equivalent to current imiglucerase dose</td>
<td>Safety</td>
</tr>
<tr>
<td>PB-06-005</td>
<td>NCT01132690</td>
<td>IV</td>
<td>12-Month, randomized, double-blind, multicenter trial</td>
<td>Pediatric patients (2 to &lt;18 years of age), ERT-naive</td>
<td>30 or 60 U/kg, every 2 weeks</td>
<td>Primary: Median percent change in hemoglobin concentration from baseline Secondary: Percent change in spleen and liver volumes, platelet count, and chitotriosidase or CCL18 activity</td>
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<tr>
<td>PB-06-006</td>
<td>NCT01411228</td>
<td>III</td>
<td>24-Month, double-blind, extension trial</td>
<td>Pediatric patients (2 to &lt;18 years of age) who completed PB-06-002 or PB-06-005</td>
<td>30 or 60 U/kg, every 2 weeks</td>
<td>Primary: Median percent change in hemoglobin concentration from baseline Secondary: Percent change in spleen, liver volume, platelet count, and chitotriosidase activity</td>
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<tr>
<td>PB-06-007</td>
<td>NCT01422187</td>
<td>III</td>
<td>21-Month, open-label extension trial</td>
<td>Adult patients with GD who completed PB-06-001 and completed PB-06-003</td>
<td>30 or 60 U/kg, every 2 weeks</td>
<td>Primary: Change from baseline in spleen volume Secondary: Change from baseline in liver volume, platelet count, and hemoglobin concentration</td>
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In summary, the future of plant cell-based production of biotherapeutics is promising because one of the fastest growing fields in the pharmaceutical industry is the need for therapeutic glycoproteins. As discussed in this review, plant-based expression systems are an attractive production platform for such proteins for many reasons. As the development of alglucerase paved the way for the production of subsequent ERTs for the treatment of GD1, the continued investigation of plant cell-expressed taliglucerase alfa will help to better define its clinical efficacy and safety in patients with GD1, and will serve as a prototypical model for development of other plant-derived biotherapeutics.

**Disclosures**

GG has intellectual property rights/patent for Synageva/Cincinnati Children’s Hospital Medical Center and Shire. He has received consulting fees from Genzyme, Pfizer and Shire. He has contracted research for Shire/HGT. MG and YS are employees of Protalix Biotherapeutics.

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**References**


