Taliglucerase alfa leads to favorable bone marrow responses in patients with type 1 Gaucher disease

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ABSTRACT

Taliglucerase alfa (Protalix Biotherapeutics, Israel) is a carrot-cell-expressed recombinant human beta-glucocerebrosidase recently approved in the United States for the treatment of type 1 Gaucher disease (GD). As bone disease is one of the most debilitating features of GD, quantification of bone marrow involvement is important for monitoring the response to treatment. Therefore, bone marrow fat fraction (Ff) measured by quantitative chemical shift imaging (QCSI) was included as exploratory parameter to evaluate bone marrow response in treatment naive GD patients participating in a double-blind, randomized phase III study. Eight GD patients with intact spleens were treated with 30 or 60 U/kg biweekly. Ff results were compared to outcomes in 15 untreated Dutch GD patients with a follow-up interval of 1 year. Five taliglucerase alfa treated patients had a Ff below the threshold that relates to complication risk (<0.23) at baseline (median (n = 8) 0.19, range 0.11–0.35). Ff significantly increased compared to baseline (p = 0.012) and compared to untreated patients (p = 0.005), already after 1 year of follow-up with further improvement up to 36 months. In four patients with the lowest Ff, the higher dose resulted in increases above 0.23 within 1 year. All patients had sustained improvements in all other parameters. There was no influence of antibodies on response parameters. Treatment with taliglucerase alfa results in significant increases in lumbar spine fat fractions, which indicates clearance of Gaucher cells from the bone marrow.

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Introduction

Gaucher disease is one of the most prevalent lysosomal storage disorders in humans. The deficient activity of the enzyme glucocerebrosidase results in accumulation of glucocerebroside in macrophages [1]. These macrophages filled with storage material, so-called Gaucher cells, accumulate mainly in the spleen, liver, and bone marrow. Type 1 Gaucher disease is the most prevalent form and is characterized by absence of central nervous system symptoms.

Skeletal disease is an important cause of disability and reduced quality of life but its pathophysiology is still poorly understood. Bone pain and acute bone crisis, (aseptic) osteomyelitis, infarcts and avascular necrosis may develop. Eventually, severe progressive and destructive bone disease necessitating joint replacement may occur in some patients [2,3]. It should be noted that the severity of cytopenia and the degree of organomegaly, other important symptoms of GD, do not always correlate with the severity of bone disease [4,5]. This necessitates separate evaluation of bone involvement to assess disease severity and risk for complications.

Enzyme replacement therapy with imiglucerase (Cerezyme™; Genzyme, MA, USA) and velaglucerase alfa (Vpriv™; Shire HGT, MA, USA) has proven to be highly effective in reversing cytopenia and reducing organ volumes [6]. In addition, long term effects with imiglucerase, which is already used for more than 15 years, indicate improvements in bone maturation in children and reductions in the frequency of avascular necrosis and nonspecific bone pain [7,8]. However, ERT cannot reverse established osseous injury, including fractures and joint collapse that occur as a result of bone infarction or local osteolysis. As bone marrow involvement precedes the occurrence of irreversible bone complications, the evaluation of treatment effects should involve sensitive techniques to detect bone marrow
infiltration. The use of plain x-rays or CT-scanning is of limited value in this respect. Magnetic resonance imaging (MRI) is regarded as the modality of choice for evaluating bone marrow disorders. In Gaucher disease, MRI can adequately pick up early bone marrow changes, before any clinical symptoms have become apparent [9]. Normal fatty marrow produces a relatively high signal intensity on T1-weighted and T2-weighted images. When adipocytes become replaced by Gaucher cells, signal intensity decreases on T1- and T2- weighted images [9,10]. Upon therapy, fatty marrow reappears, resulting in gradual increase in signal intensities on T1- and T2- weighted images [11]. This technique has also been applied to compare dose effects. Indeed, bone marrow involvement as assessed by MRI improved faster and more pronounced in the higher-dosed group [12].

For better quantification of bone marrow fat fractions, quantitative chemical shift imaging (QCSI) has been investigated and found to be significantly lower in Gaucher disease patients compared to the healthy population and to increase upon treatment [11,13,14]. Mean values reported for the healthy adult population in these studies vary from 0.29 [13] to 0.37 [15] and are age dependent. Bone complications primarily occur in patients with a bone marrow fat fraction <0.23) and univariate logistic regression analysis revealed that for every decrease of Ff of 0.1 the risk for the occurrence of bone complications increased with 85% [16]. Histological studies have revealed that infiltration with Gaucher cells is associated with a decrease in adipocytes [17] and that enzyme replacement therapy results in decrease in Gaucher cells and restoration of fat and normal hematopoietic tissue [18].

Although variations occur and untreated patients with a low fat fraction may remain asymptomatic for many years [19], it is believed that in general, increased invasion of the bone marrow with Gaucher cells creates a higher risk for avascular necrosis, infarcts and crises [20]. Bone marrow evaluations have therefore become part of international guidelines [21,22] and are being employed during investigations for new treatments.

Taliglucerase alfa (Elelyso™, Protalix Biotherapeutics, Carmiel, Israel/Pfizer Inc., New York, USA) is a carrot-cell-expressed recombinant human α-glucocerebrosidase, which has recently been approved by the FDA as a new enzyme replacement therapy for patients with Gaucher disease. A phase III double-blind, randomized, parallel dose groups (30 U/kg biweekly and 60 U/kg biweekly) clinical trial was completed. The results of this 9-month, 20-infusion clinical trial (NCT number: 00376168) showed statistically significant improvements compared with baseline in spleen and in all secondary efficacy endpoint measures (liver volumes, hemoglobin levels, and platelet counts), with a faster response for the 60 U/kg dose [23]. Bone marrow fat fraction (FF) measured by quantitative chemical shift imaging (QCSI) was included as exploratory parameter in this pivotal trial to evaluate bone marrow response in a subpopulation of the GD patients participating in this trial. The current paper describes FF responses to taliglucerase alfa up to 36 months of therapy as well as their relations to the overall responses in these patients. The FF data are compared to outcomes in untreated patients, extracted from the AMC Gaucher database.

Material and methods

Taliglucerase alfa treated patients

Eight of the 10 patients from the aforementioned pivotal trial [23] who agreed to undergo exploratory analysis of FF at the AMC in Amsterdam continue into an extension study long term follow up protocol. Selection was made solely on the basis of agreeing to travel to Amsterdam.

Four received 30 U/kg every other week (the low-dose group) and four received 60 U/kg every other week (high-dose group). FF results at baseline and after 12, 24 and 36 months after the start of treatment were available for all 8 patients. FF results after 9 months were available in 7 of 8 patients (missing in one patient due to a technical error).

Untreated control patients

Data from these 8 patients were compared to untreated control patients. Results of 15 untreated Dutch Gaucher patients were included for a comparison of FF response after 12 months (median 12.2, range 8.4–14 months). Of these 15 patients, in 9 patients results after 24 months of untreated follow up were available (median 24.1, range 19.1–28.3 months) and in 6 patients results after 36 months of untreated follow up were available (median 36, range 33.5–41.4).

Bone marrow fat fractions

MRI was performed on a General Electric Signa Horizon MR Machine until August 2009 and on a Siemens Avanto Machine since then. Signal is acquired with a 2-point Dixon spin-echo sequence (TE/TR: 22/2500) and the FF is calculated using a homemade algorithm [24].

Scanner change has been shown to have no systematic effect [25]. Special effort is taken to (re-)position the image slice on midsagittal localizer images for each consecutive measurement in the same patient as accurately as possible and to obtain regions of interest of L3, L4 and L5 in a standardized manner. When performed according to this protocol reproducibility (SD of repeated measurements) is 0.01–0.03 in Ff [15].

Correlation with overall response

To study the relation between the response in FF measurements and the overall response to treatment with taliglucerase alfa the following parameters were included:

- Haemoglobin level and platelet count
- Liver and spleen volumes as assessed by MRI
- Chitotriosidase activity as measured using the 4MU-deoxychitobioside substrate, as described by Aguilera et al. [26] and slightly modified by Schoonhoven et al. [27].

Data on chitotriosidase activity were only available up to 24 months of follow-up.

Antibody assessment

Antibodies to taliglucerase alfa were determined using an ELISA based anti-drug antibody (ADA) assay according to draft FDA guidance.

ADA positive patient's samples were further characterized for neutralizing activity using an anti-taliglucerase alfa antibody as a positive control. One assay is an in-vitro neutralizing assay where (reduction of) enzyme activity is measured in the presence of patient serum. The second assay is a cell based assay which measured the level of active taliglucerase alfa taken up by macrophage cells following incubation of cultured macrophages with taliglucerase alfa in the presence of patient serum. This assay coupled both the detection of reduced uptake of the drug into target cells and reduced intracellular activity of the drug. A reduced intracellular activity is measured in the event that taliglucerase alfa and ADA complexes interfere with either cellular uptake or intracellular enzyme activity.

Statistical methods

For statistical calculations PASW statistics 18 software package was used.

Absolute changes in bone marrow fat fractions were calculated. Different groups were compared for bone marrow fat fraction response. Comparisons were made between patients treated with taliglucerase alfa irrespective of dose and untreated patients. In addition comparisons
were made between taliglucerase alfa 30 U/kg biweekly and taliglucerase alfa 60 U/kg biweekly and untreated patients.

Differences in Ff compared to baseline in taliglucerase alfa treated patients were tested using the Wilcoxon signed rank test. Differences between treated and untreated groups as well as between different dosing groups with respect to absolute change in Ff values were tested for statistical significance using the Mann–Whitney U test.

Results

Institutional review board approval for the pivotal trial was obtained at each site (Jerusalem, Haifa, Belgrade) [23]. All participants gave written informed consent.

Table 1 describes the characteristics of the patients.

Five taliglucerase alfa treated patients had a baseline Ff <0.23. Median Ff at baseline (n = 8) was 0.19 (range 0.11–0.35) (see Table 2).

All taliglucerase alfa treated patients showed an increase in Ff, with median absolute changes compared to baseline of 0.10 (range 0.03–0.19) after 1 year of treatment, 0.095 (range 0–0.28) after 2 years of treatment and 0.135 (range 0.05–0.29) after 3 years of treatment, see Table 2. Results after 9 months (n = 7) of 8) show an increase in Ff, which is comparable to the response seen after 1 year.

Taliglucerase alfa treated patients were divided according to their dose: 30 and 60 U/kg biweekly and compared to the group of untreated patients. Ff significantly increased compared to baseline (p = 0.012) and compared to untreated patients (n = 15, p 0.005), already after 1 year of follow-up with further improvements up to 36 months. Fig. 1 depicts the median Ff values over 36 months of follow-up for the taliglucerase alfa treated patients as compared to the subgroup of six untreated patients for whom results up to 36 months of untreated follow-up were available.

Dose did not have a significant influence, but for the four patients with the lowest Ff (pts nr. 1, 2, 7 and 8 in Table 1) the two treated with the higher dose (pts nr. 7 and 8) had an increase above 0.23 already in 1 year, while this took longer in the two patients treated with 30 U/kg biweekly.

Color coded images of a typical example of Ff results in an untreated patient and a taliglucerase alfa treated patient are shown in Fig. 2.

Relation with overall response

Individual results of bone marrow fat fractions and other disease parameters are shown in Fig. 3.

Patients 1 (subject 10–001 from the pivotal trial) and 2 (10–028) showed relatively moderate increases in Ff in response to treatment. Despite baseline values <0.23, the absolute changes in these patients were respectively 0.10 and 0.08 compared to baseline.

Ff increased to values >0.23 in patient 1, but the increase is modest compared to patients 7 and 8 with comparable baseline Ff. This corresponds to an overall more moderate response seen in this patient, despite a clear decline in spleen volume, decreasing from 21 Multiples of Normal (MN) to 12 MN after 36 months of treatment.

Table 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex (fm)</th>
<th>Age (median, range)</th>
<th>Spleen status (Sx:no Sx)</th>
<th>Ff at baseline (median, range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taliglucerase alfa treated patients, 30 U/kg biweekly</td>
<td>2:2</td>
<td>34 (25–35)</td>
<td>0:4</td>
<td>0.19 (0.11–0.23)</td>
</tr>
<tr>
<td>Taliglucerase alfa treated patients, 60 U/kg biweekly</td>
<td>1:3</td>
<td>46 (33–54)</td>
<td>0:4</td>
<td>0.235 (0.13–0.35)</td>
</tr>
<tr>
<td>Untreated patients</td>
<td>9:6</td>
<td>40 (18–76)</td>
<td>3:12</td>
<td>0.24 (0.13–0.57)</td>
</tr>
</tbody>
</table>

Patient 2 is the only patient within this cohort that does not show an increase in Ff to values >0.23 within 36 months of treatment. Similar observations have been made in some patients treated with imiglucerase (personal experience). However, overall response in this patient is satisfactory with (near) normalisation of platelet count and a sharp decrease in chitotriosidase activity (after 24 months) and (more modest) decreases in organ volumes.

Antibody formation and adverse events

Seven patients were positive for IgG antibodies (3 patients treated with 30 U/kg biweekly and 4 treated with 60 U/kg biweekly) of whom 2 tested positive for neutralizing activity (patients 4 and 6) in the neutralizing assay in serum, but none in the cell-based assay. Patient 3 is the only patient in whom antibody tests remained negative throughout the study. Patient 8 tested positive for IgG antibodies at the fourth infusion, but no antibodies could be shown in subsequent visits. The presence of antibodies had no negative influence on the outcome of any of the parameters.

A total of 112 adverse events (AEs) were reported among 8 patients of which 20 AEs were reported as treatment related. These include 1 patient with a hypersensitivity reaction at infusion 23, one patient who displayed an intermittent fixed drug eruption after 15 months which last appeared 14 months after this first infusion and one patient with infusion related reactions (dizziness, chills, nausea and pain at the infusion site). Both patients continue treatment with taliglucerase alfa, the patient experiencing the hypersensitivity reaction at a slower infusion rate combined with premedication (loratadine and ranitidine 12 and 2 h before the infusion).

Discussion

The current study shows that treatment with taliglucerase alfa results in significant increases in fatty marrow, reflecting clearance of Gaucher cells in patients with type I Gaucher disease. Compared to untreated patients, Ff increased significantly, corresponding to the overall satisfactory response seen in the taliglucerase alfa treated patients.

Although for the final Ff responses no statistical difference could be established between 30 and 60 U/kg biweekly, the patients with the lowest Ff in the high dose group had a faster response compared to the two patients with the lowest Ff in the low dose group. While this is suggestive of a dose effect, it is also noteworthy that the two patients displaying a fast response are the oldest patients in the cohort. As Ff increases with age, the low baseline fat fraction in these two patients could reflect more severe infiltration (as the expected Ff corresponding to their age is higher) with a subsequent faster response.

An increase to a level >0.23 was achieved for 7/8 patients. This cut off level has been used in the past as an indication for “bone at risk,” based upon earlier studies at the AMC [16]. One patient in the lower dose group (P2) did not yet reach this threshold after 36 months of treatment. A slow bone marrow response usually simply reflects the variation in responses that can occur and more exceptionally can be attributed to a general slow or absent response as a result of neutralizing antibodies or possibly local abnormalities such as fibrosis in the marrow. Since this patient showed adequate reductions in liver and spleen size, and a favorable chitotriosidase response, it is unlikely that the presence of antibodies had an effect. In addition, no neutralizing activity for the antibodies in this patient was shown. So far, we have not been able to identify specific predictors for poor responders [4].

Two studies have previously reported on the response of Ff to enzyme replacement therapy with imiglucerase [8,14]. Rosenthal et al. investigated the response in 11 of 12 patients after 42 months of treatment at initial dosages of 60 U/kg biweekly and found a median
patients after 48 months of follow-up of 3 years (n = 6) are included in these graphs for each time point.

Table 2

<table>
<thead>
<tr>
<th>Nr.</th>
<th>Sex</th>
<th>Age</th>
<th>Genotype</th>
<th>Dose (U/kg biweekly)</th>
<th>Ff at baseline</th>
<th>Ff after 9 months</th>
<th>Ff after 12 months</th>
<th>Ff after 24 months</th>
<th>Ff after 36 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1 (10-001)</td>
<td>M</td>
<td>25</td>
<td>N370S/N370S</td>
<td>30</td>
<td>0.16</td>
<td>X</td>
<td>0.20</td>
<td>0.16</td>
<td>0.26</td>
</tr>
<tr>
<td>P2 (10-028)</td>
<td>M</td>
<td>35</td>
<td>N370S/844G</td>
<td>30</td>
<td>0.11</td>
<td>0.17</td>
<td>0.19</td>
<td>0.20</td>
<td>0.19</td>
</tr>
<tr>
<td>P3 (15-015)</td>
<td>F</td>
<td>35</td>
<td>N370S/N370S</td>
<td>30</td>
<td>0.23</td>
<td>0.24</td>
<td>0.26</td>
<td>0.31</td>
<td>0.34</td>
</tr>
<tr>
<td>P4 (30-008)</td>
<td>F</td>
<td>33</td>
<td>N370S/c.1265_1319del</td>
<td>30</td>
<td>0.22</td>
<td>0.39</td>
<td>0.38</td>
<td>0.39</td>
<td>0.40</td>
</tr>
<tr>
<td>P5 (10-005)</td>
<td>F</td>
<td>33</td>
<td>N370S/N370S</td>
<td>60</td>
<td>0.35</td>
<td>0.42</td>
<td>0.38</td>
<td>0.38</td>
<td>0.40</td>
</tr>
<tr>
<td>P6 (15-016)</td>
<td>M</td>
<td>41</td>
<td>N370S/N370S</td>
<td>60</td>
<td>0.33</td>
<td>0.43</td>
<td>0.45</td>
<td>0.42</td>
<td>0.49</td>
</tr>
<tr>
<td>P7 (30-009)</td>
<td>M</td>
<td>52</td>
<td>N370S/R463H</td>
<td>60</td>
<td>0.14</td>
<td>0.27</td>
<td>0.28</td>
<td>0.37</td>
<td>0.40</td>
</tr>
<tr>
<td>P8 (30-011)</td>
<td>M</td>
<td>54</td>
<td>N370S/D409H, H255Q</td>
<td>60</td>
<td>0.13</td>
<td>0.30</td>
<td>0.32</td>
<td>0.41</td>
<td>0.42</td>
</tr>
</tbody>
</table>

Fig. 1. Changes in bone marrow fat fractions for all groups. Median and range are presented. Of the untreated AMC cohort, only those patients that had an untreated follow-up of 3 years (n = 6) are included in these graphs for each time point.
L.v.D. received grants and/or support for travel to meetings for the study.

A.Z. receives consultancy fees from, and has share options in, Protalix Biotherapeutics and sits on their scientific advisory board.

E.A. declares no competing financial interests.

J.A. received reimbursements of travel costs and honoraria for participation in symposia from Genzyme and Shire. All honoraria are donated to research funds at the AMC.

M.P. and H.R. received grants and/or support for travel to meetings for the study, and provision of medicine, equipment, and administrative support was given to each trial site.

D.E. received reimbursements of costs for participation in meetings and conferences by Shire HGT.

D.A., E.B.-A. and R.C. are employees of Protalix Biotherapeutics. D.A. is the president and chief executive officer of Protalix Biotherapeutics.

E.B.-A. is a vice president of Protalix Biotherapeutics. R.C. is the vice president of medical affairs of Protalix.

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