Sebelipase alfa over 52 weeks reduces serum transaminases, liver volume and improves serum lipids in patients with lysosomal acid lipase deficiency

Vassili Valayannopoulos1, Vera Malinova2, Tomas Honzik2, Manisha Balwani3, Catherine Breen4, Patrick B. Deegan5, Gregory M. Enns6, Simon A. Jones4, John P. Kane7, Eveline O. Stock8, Radhika Tripathani9, Stephen Eckert9, Eugene Schneider9, Gavin Hamilton10, Michael S. Middleton10, Claude Sirlin10, Bruce Kessler11, Christopher Bourdon12, Simeon A. Boyadjiev13, Reena Sharma14, Chris Twelves15, Chester B. Whitley16, Anthony G. Quinn9,*

1Ref Centre IEM, Necker-Enf Malades Hosp, IMAGINE Institute and Paris Descartes University, Paris, France; 2Department of Pediatrics, 1st Faculty of Medicine, Charles University and General University Hospital, Prague, Czech Republic; 3Department of Genetics and Genomic Sciences, The Mount Sinai School of Medicine, New York, NY, United States; 4Manchester Centre for Genomic Medicine, St. Mary's Hospital, Manchester, UK; 5Department of Medicine, Addenbrooke's Hospital, Cambridge, UK; 6Department of Pediatrics, Lucile Packard Children's Hospital, Stanford University, Stanford, CA, United States; 7Divisions of Endocrinology & Metabolism, University of California, San Francisco, CA, United States; 8Divisions of Cardiology, University of California, San Francisco, CA, United States; 9Synageva BioPharma Corp., Lexington, MA, United States; 10University of California, San Diego, CA, United States; 11Eureka Internal Medicine, Eureka, CA, United States; 12Health Sciences North, Sudbury, Ontario, Canada; 13University of California, Davis Medical Center, Department of Pediatrics, Section of Genetics, Davis, CA, United States; 14Salford Royal NHS Foundation Trust, Salford, UK; 15Leeds Institute of Cancer and Pathology, Leeds, UK; 16University of Minnesota, Minneapolis, MN, United States

Background & Aims: Lysosomal acid lipase deficiency is an autosomal recessive enzyme deficiency resulting in lysosomal accumulation of cholesteryl esters and triglycerides. LAL-CL04, an ongoing extension study, investigates the long-term effects of sebelipase alfa, a recombinant human lysosomal acid lipase.

Methods: Sebelipase alfa (1 mg/kg or 3 mg/kg) was infused every-other-week to eligible subjects. Safety and tolerability assessments, including liver function, lipid profiles and liver volume assessment, were carried out at regular intervals.

Results: 216 infusions were administered to eight adult subjects through week 52 during LAL-CL04. At week 52, mean alanine aminotransferase and aspartate aminotransferase levels were normal with mean change from baseline of −58% and −40%. Mean changes for low-density lipoprotein, total cholesterol, triglyceride and high-density lipoprotein were −60%, −39%, −36%, and +29%, respectively. Mean liver volume by magnetic resonance imaging and hepatic proton density fat fraction decreased (12% and 55%, respectively). Adverse events were mainly mild and unrelated to sebelipase alfa. Infusion-related reactions were uncommon: three events of moderate severity were reported in two subjects; one patient’s event was suggestive of a hypersensitivity-like reaction, but additional testing did not confirm this, and the subject has successfully re-started sebelipase alfa. Of samples tested to date, no anti-drug antibodies have been detected.

Conclusions: Long-term dosing with sebelipase alfa in lysosomal acid lipase-deficient patients is well tolerated and produces sustained reductions in transaminases, improvements in serum lipid profile and reduction in the hepatic fat fraction. A randomized, placebo-controlled phase 3 trial in children and adults is underway (ARISE: NCT01757184).

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Introduction

Lysosomal Acid Lipase (LAL) Deficiency (LALD) (OMIMD 278000) is an autosomal recessive disease that is associated with significant morbidity and shortened life expectancy. Mutations in the LIPA gene markedly decrease LAL enzyme activity leading to
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lysosomal cholesteryl ester (CE) and triglyceride (TG) accumulation. Although the LIPA gene is expressed in many tissues, lysosomal accumulation of undigested lipids is prominent in cells of the monocyte/macrophage lineage, in the liver and hepatocytes [1]. Common clinical manifestations include serum transaminase elevation, hepatomegaly, hepatic lipid accumulation, and dyslipidemia. This presentation, historically known as cholesteryl ester storage disease, is an underappreciated cause of liver fibrosis with frequent progression to cirrhosis [2]. LAL D is also associated with evidence of premature atherosclerosis in some cases [3–10]. Clinical diagnosis is challenging due to the prevalence (1:40,000 to 1:300,000 [3,11]) and manifestations that overlap with more common liver/lipid disorders.

In contrast to non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), where the pathogenesis is not fully understood, LAL D leads to CE and TG accumulation in hepatocytes and liver macrophages with progression to fibrosis. The high frequency of liver fibrosis with cirrhosis development in LAL D, for some as early as 6 months of age, suggests that the accumulation of lysosomal CE and TG is a potent driver of liver fibrosis [2,12–14]. In the rat disease model of LAL D, liver fibrosis also develops rapidly (within 4–8 weeks) in association with abnormal lipid accumulation. Concordant reduction in liver CE, TG, alpha smooth muscle actin staining and fibrosis with sebelipase alfa (a recombinant human LAL enzyme; Synageva BioPharma Corp., Lexington, MA, US) highlights the importance of lysosomal CE and TG accumulation as a driver of fibrosis [15].

Current medical management of LAL D is limited and includes the use of HMG-CoA reductase inhibitors (statins) alone or in combination with other lipid-lowering therapies for disease-associated hypercholesterolemia. Although these agents can reduce serum cholesterol and TG concentrations, these changes are not accompanied by consistent improvements in serum transaminases or substantial reductions in hepatic CE or TG content [2,16]. These findings, and the observed decreases in stellate cell activation and fibrosis concordant with hepatic lipid reduction in the rat model, point to the importance of hepatic lipid reduction in the amelioration of liver disease progression in these patients.

The initial effects of sebelipase alfa in LAL D adults in the LAL-CL01 study and effects up to 12 weeks in the LAL-CL04 study have been reported [17]. We now provide evidence of these beneficial effects on biochemical markers of disease activity up to week 52, describe for the first time improvements in hepatic lipid content, and additionally report longer term safety.

Patients and methods

Study design

LAL-CL04 (trial registration number: NCT01488097) is an ongoing open-label, multicentre, extension study of LAL-CL01 (NCT01307098) involving eight sites in five countries. Subjects who completed the LAL-CL01 study were eligible to enrol in this extension study (Fig. 1). The dose schedule in the LAL-CL04 study consisted of four once-weekly infusions of sebelipase alfa at the same dose as in the LAL-CL01 study (0.35, 1.0 or 3.0 mg/kg) followed by every-other-week infusions of sebelipase alfa (1.0 or 3.0 mg/kg).

The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki and Good Clinical practice guidelines. Ethics committees and/or institutional review boards at participating institutions reviewed and approved the protocol. All subjects provided informed written consent before undergoing study-specific assessments or procedures.

Investigations

The objectives of LAL-CL04 were to evaluate the long-term safety, pharmacokinetics, pharmacodynamics, and immunogenicity of sebelipase alfa. Pharmacokinetic and clinical effects were assessed by measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), total cholesterol, TGs, low-density lipoprotein (LDL), high-density lipoprotein (HDL), alkaline phosphatase, gamma-glutamyl transferase (GGT), C-reactive protein and ferritin. Liver volume was assessed by MRI and the hepatic proton density fat fraction (PDFF), a measure of lipid content, was assessed by MRI (multi-echo gradient-echo sequence imaging) or 1H-MRS (if available) [18–21]. Safety assessments included treatment-related adverse events (AEs), vital signs, physical examination, electrocardiography, and routine laboratory tests at regular intervals. AEs were graded using the National Cancer Institute Common Terminology Criteria for adverse events (CTCAE), version 4.0 or higher. Infusion-related reactions (IRRs) were defined as any AE that occurred during the 2-h infusion or within 4 h after the infusion and assessed by the investigator as at least possibly related to the study drug.

Anti-drug antibody assays

The presence of serum anti-sebelipase alfa antibodies was examined by use of a validated bridging enzyme-linked immunosorbent assay [22,23]. Additional methodological details have been published previously [17].

Statistical analysis

Subjects who received at least one dose of sebelipase alfa in LAL-CL04 were analysed for safety; AEs, vital signs, and laboratory tests were summarized. Statistical comparisons of the dosing cohorts were not performed, as the study was not powered to detect differences between them. Data from the two cohorts were pooled and descriptive statistics were used to compare to baseline parameters from LAL-CL01. Changes and percent changes from baseline in serum transaminases, serum lipids, acute phase reactants, hepatic PDFF and liver volumes were summarized with no specific statistical hypothesis testing. Exploratory statistical analyses were performed to examine the effects of sebelipase alfa on key activity parameters. The Wilcoxon sign-rank test was used for statistical tests of change from baseline, without adjustment for multiplicity. For each measure, only the p value for the absolute change from baseline was calculated; this p value was used to describe the change from baseline regardless of the summary statistic that is displayed (i.e., change from baseline or percentage change from baseline).

Starting with the week 4 visit, laboratory assessments were performed two weeks post-infusion. In order to further investigate the post-infusion increases in LDL and TG an additional serum biochemistry assessment was scheduled one week post-infusion (between week 24 and 28). In the graphs, all 1-week post-infusion laboratory data are presented one week after the week 24 visit. For end points calculated as change from baseline, the LAL-CL01 study baseline was used, with the exception of the liver volume and hepatic PDFF end points, which were first performed using MRI/MRS at baseline of LAL-CL04. Liver volume is displayed as multiples of normal (MN, where “normal” liver volume in litres was defined as 2.5% of body weight in kg). Hepatic PDFF is reported for the right lobe of the liver using either MRI (n = 4 subjects) or MRS (n = 2 subjects). Percentage change from baseline was computed for all six subjects and combined to form the mean percentage change in hepatic PDFF.

Results

The first subject entered LAL-CL04 on 12 December 2011; the last subject completed the last visit on 15 May 2013. Of the nine subjects who completed LAL-CL01, eight enrolled in LAL-CL04 with the ninth subject (#4) not enrolling. The patient completed the last visit on 15 May 2013. Of the nine subjects who completed LAL-CL01, eight enrolled in LAL-CL04 with the ninth subject (#4) not enrolling. The patient completed LAL-CL04, but was subsequently lost to follow-up. When the patient made contact with the trial centre again approximately 9 months later, the patient had already developed liver failure, manifested by oedema, fatigue and grade 1 hepatic encephalopathy. The patient was no longer eligible for
Fig. 1. Flow chart diagram of the LAL-CL01 and LAL-CL04 study design. Subjects (18–65 yr) were required to have documented LAL deficiency, hepatomegaly and/or transaminases >1.5 × ULN, and may be on a lipid-lowering agents (stable dose for >4 weeks). LAL, Lysosomal acid lipase; qw, once weekly; qow, once every other week.

Table 1. Baseline demographic and disease characteristics of all subjects in LAL-CL04.

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2*</th>
<th>3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>LAL-CL01 dose (mg/kg)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>LAL-CL04 dose (Wk 1 to 4) (mg/kg)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>LAL-CL04 dose (Wk 6 to 52) (mg/kg)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
</tbody>
</table>

Demographics

Baseline age (yr) | 41 | 29 | 27 | 45 | 19 | 21 | 19 | 41 | 30 ± 11
Gender | M | M | F | M | M | F | M | F | 75% | M
Baseline BMI | 27.8 | 22.6 | 20.5 | 25.1 | 23.5 | 25.2 | 26.6 | 27.4 | 24.8 ± 2.5

Baseline laboratory assessments

ALT (U/L) | 60 | 76↑ | 85↑ | 70↑ | 119↑ | 86↑ | 57 | 110↑ | 83 ± 22
AST (U/L) | 56↑ | 48 | 69↑ | 52↑ | 69↑ | 41 | 37 | 65↑ | 53 ± 12
GGT (U/L) | 40 | 34 | 12 | 42 | 26 | 55 | 23 | 203↑ | 54 ± 61
Total cholesterol (mg/dl) | 130 | 391↑ | 256↑ | 116 | 178 | 188 | 182 | 220 | 169 ± 39
HDL-cholesterol (mg/dl) | 28↑ | 41 | 39 | 22↑ | 45 | 43 | 49 | 26↑ | 40 ± 9
LDL-cholesterol (mg/dl) | 74 | 300↑ | 208↑ | 70 | 118 | 123 | 135 | 143 | 145 ± 76
Triglycerides (mg/dl) | 218↑ | 108 | 106 | 102 | 92 | 266↑ | 80 | 277↑ | 147 ± 85
ALP (U/L) | 93 | 97 | 76 | 86 | 135↑ | 100 | 76 | 61 | 84 ± 14

Serum ferritin (ng/ml)

Male | 221 | 300 | - | 283 | 182 | 283 | - | 342 | 269 ± 58
Female | - | - | 118 | - | - | 89 | - | 104 ± 21

Baseline LAL activity in leukocytes (μmol/g/h) | 42↑ | 42↑ | 33↑ | 57↑ | 10↑ | 42↑ | 24↑ | 19 | 34 ± 15
Baseline hepatomegaly (PE) | Yes | Yes | Yes | Yes | No | Yes | Yes | Yes | 88%

Lipid-lowering medications

Yes | No | Yes* | Yes | No | No | Yes | 75% | Yes
Yes | No | No* | No | No | No | Yes | Yes | 63%
Yes | No | Yes* | No | Yes | No | No | No | 25%
Yes | No | No | Yes | No | No | No | No | 25%

Liver volume (MN) | 1.36 | 1.15 | 0.87 | 1.30 | 1.02 | 1.01 | 0.98 | 0.99 | 1.09 ± 0.17

Hepatic PDFF (%)** | n.a. | 12.29 | 10.41 | 6.00 | 8.03 | 4.00 | 11.05 | 9.00 | 8.68 ± 2.92

Subject numbers were allocated at enrolment to LAL-CL01. Subject #4 did not progress to study LAL-CL04 and therefore was not included in this table. Baseline is defined as date of first dose. All demographic and baseline data were assessed prior to first infusion in study LAL-CL01, with the exception of liver volume and hepatic PDFF, which were assessed prior to the first infusion in LAL-CL04.

BMI, body mass index; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma glutamyl transpeptidase; LDL, low-density lipoprotein; HDL, high-density lipoprotein; MN, multiples of normal, where “normal” liver volume in liters is defined as 2.5% of body weight in kg; PDFF, proton density fat fraction; PE, physical examination.

Above central lab upper limit of normal.

Below central lab lower limit of normal.

*Subject discontinued medications in between study LAL-CL01 and LAL-CL04.

**Four subjects had baseline hepatic PDFF assessed by MRI (multi-echo gradient-echo sequence imaging), two by MRS.
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LAL-CL04, and was referred to the trial centre for liver transplant assessment. The patient was listed for urgent orthotopic liver transplant and after two months, liver transplant was carried out successfully; the patient is currently well.

The data presented include seven subjects treated through to week 52 and one subject (#2) treated through to week 38.

Demographics and baseline disease characteristics

Demographic and baseline characteristics are described in Table 1. All subjects were Caucasian and 75% were male. The mean age at first dose for the eight subjects who entered LAL-CL04 was 31 ± 11 years. Six subjects were receiving treatment with lipid-modifying therapies, including statins, ezetimibe, and other medications; despite this, only two of the subjects’ lipid profiles were within the central laboratory reference range prior to entering the study. Subject #3 discontinued lipid-lowering medication between studies and had a higher baseline LDL in LAL-CL04 than in LAL-CL01. This subject re-started lipid-lowering medications at week 39 (see the serum lipids section).

Clinical assessments

Serum transaminases

As previously reported, initiation of treatment with sebelipase alfa produced a rapid decline in ALT and AST (Fig. 2). Six subjects had abnormal transaminases at baseline, and all normalized upon treatment. A transient increase in mean AST was noted at week 24/25 due to an isolated increase in AST in a single subject from 42 U/L to 277 U/L. No obvious cause for this transient increase, including alcohol consumption or medication change, was identified, and testing repeated one week later revealed that the subject’s AST had returned to 44 U/L. The overall improvements in ALT and AST were sustained through the week 38 and 52 time points. At week 52, sebelipase alfa treated–subjects had a mean concentration of ALT and AST of 34 U/L and 32 U/L, respectively. Values were within the central laboratory normal reference ranges and the mean percentage decreases from LAL-CL01 baseline were 58% and 40%, respectively (p = 0.016; n = 7). There was no clear evidence of a dose-related effect in the time-to-onset or in the magnitude of the reduction in AST and ALT (Table 2). In subject #2 for whom treatment was paused at week 38, AST and ALT were decreased from LAL-CL01 baseline at all the visits prior to pausing treatment.

Serum lipids

All seven subjects who received treatment with sebelipase alfa through week 52 showed decreases from their original LAL-CL01 baseline concentrations of total cholesterol, LDL, and increases in HDL, while six showed decreases in TGs. On average, LDL decreased by 73 ± 31 mg/dl (60%, p = 0.016), total cholesterol by 71 ± 31 mg/dl (39%, p = 0.016) and TGs by 72 ± 66 mg/dl (36%, p = 0.047). Additionally, an increase in HDL by 9 ± 6 mg/dl (29%, p = 0.031) was observed (Fig. 3). The beneficial effects of sebelipase alfa on the lipid profile seen in LAL-CL01 up to week 12 were also evident across week 12 to 52. For LDL and HDL there was evidence of continued improvement over time with additional improvements in these parameters between weeks 38 and 52. For TGs, two of the three subjects on the highest dose had notably large reductions, but these subjects also had the highest baseline TG levels, and thus dose and baseline TG levels were confounded.

Lipid-lowering therapy (combination ezetimibe 10 mg/simvastatin 20 mg po qd) had been discontinued in subject #3 between completing LAL-CL01 and beginning of LAL-CL04. At week 39, this subject resumed therapy with simvastatin (20 mg po qd). There was evidence that the statin or statin/ezetimibe combination with sebelipase alfa in this subject had an additive effect on the reduction in LDL concentrations seen with sebelipase alfa.

MR-measured hepatic PDFF and liver volumes

 Imaging performed at weeks 10 or 12, 24, and 52 demonstrated a mean decrease in liver volume (MN) from the LAL-CL04 baseline of 10%, 9%, and 12% (n = 8 at weeks 10 and 24, n = 7 at week 52). Additionally, a mean relative reduction in the hepatic proton density fat fraction (PDFF) from LAL-CL04 baseline of 42%, 35%, and 55%, respectively, was observed (n = 7 at weeks 10 and 24, n = 6 at week 52) (Fig. 5).

Dose effects

Graphical exploration of the individual subject changes in the end points did not reveal any apparent trends by dose cohort (Table 2); however, the sample size in each dose group was small, and thus the ability to detect dose differences was limited. For the 6 subjects who received their full dosing regimen, the cumulative dose (across LAL-CL01 and LAL-CL04) at LAL-CL04 week 52 was 26.8 mg/kg, 32 mg/kg, and 96 mg/kg for subjects initially assigned to 0.35 mg/kg, 1.0 mg/kg, and
3.0 mg/kg doses, respectively. The potential dose effects were graphically explored and no apparent trends were observed.

**Safety assessment**

216 infusions of sebelipase alfa were administered from week 12 to week 52 in LAL-CL04. Subject #2 experienced CTCAE grade 2 (moderate) hyperaemia and chills 30 min into his week 36 infusion and a similar reaction during his week 38 infusion, despite pre-treatment with diphenhydramine and acetaminophen. The week 38 infusion-reaction worsened after discontinuation of the infusion and administration of intravenous antihistamines. Although no changes were noted in the subject’s heart rate, blood pressure and oxygen saturation during this reaction the subject reported throat tightness and was administered subcutaneous

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### Table 2. Absolute and relative change from LAL-CL01 baseline in laboratory values at week 52 of LAL-CL04.

<table>
<thead>
<tr>
<th>Subject</th>
<th>1</th>
<th>2*</th>
<th>3</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>Mean ± SD</th>
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</thead>
<tbody>
<tr>
<td>LAL-CL01 dose (mg/kg)</td>
<td>0.35</td>
<td>0.35</td>
<td>0.35</td>
<td>1</td>
<td>1</td>
<td>3</td>
<td>3</td>
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<tr>
<td>LAL-CL04 dose (Wk 1 to 4) (mg/kg)</td>
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<td>0.35</td>
<td>0.35</td>
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</tr>
<tr>
<td>LAL-CL04 dose (Wk 6 to 52) (mg/kg)</td>
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<td>1</td>
<td>1</td>
<td>1</td>
<td>3</td>
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### Demographics

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<th>27</th>
<th>45</th>
<th>19</th>
<th>21</th>
<th>19</th>
<th>41</th>
<th>30 ± 11</th>
</tr>
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<tbody>
<tr>
<td>ALT (U/L)</td>
<td>34 (-26)</td>
<td>-</td>
<td>32 (-53)</td>
<td>29 (-41)</td>
<td>50 (-69)</td>
<td>52 (-34)</td>
<td>18 (-39)</td>
<td>26 (-84)</td>
<td>-49 ± 21</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>45 (-11)</td>
<td>-</td>
<td>36 (-33)</td>
<td>28 (-24)</td>
<td>30 (-39)</td>
<td>30 (-11)</td>
<td>21 (-16)</td>
<td>37 (-28)</td>
<td>-23 ± 11</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>12 (-28)</td>
<td>-</td>
<td>8 (-4)</td>
<td>25 (-17)</td>
<td>14 (-12)</td>
<td>43 (-12)</td>
<td>12 (-11)</td>
<td>93 (-110)</td>
<td>-28 ± 37</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>65 (-65)</td>
<td>-</td>
<td>137 (-119)</td>
<td>69 (-47)</td>
<td>121 (-57)</td>
<td>131 (-57)</td>
<td>141 (-41)</td>
<td>108 (-112)</td>
<td>-71 ± 31</td>
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<tr>
<td>HDL-cholesterol (mg/dl)</td>
<td>33 (5)</td>
<td>-</td>
<td>48 (9)</td>
<td>32 (10)</td>
<td>46 (1)</td>
<td>49 (6)</td>
<td>63 (14)</td>
<td>45 (19)</td>
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<td>LDL-cholesterol (mg/dl)</td>
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<td>-</td>
<td>79 (-129)</td>
<td>22 (-48)</td>
<td>58 (-60)</td>
<td>62 (-50)</td>
<td>70 (-65)</td>
<td>40 (-103)</td>
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<td>Triglycerides (mg/dl)</td>
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<td>63 (-39)</td>
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<td>131 (-135)</td>
<td>102 (22)</td>
<td>166 (-111)</td>
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<td>ALP (U/L)</td>
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<td>-</td>
<td>57 (-19)</td>
<td>60 (-28)</td>
<td>83 (-52)</td>
<td>89 (-11)</td>
<td>60 (-16)</td>
<td>38 (-23)</td>
<td>-21 ± 16</td>
</tr>
<tr>
<td>Serum ferritin (ng/ml)</td>
<td>92 (-129)</td>
<td>-</td>
<td>-</td>
<td>158 (-125)</td>
<td>160 (-22)</td>
<td>144 (-139)</td>
<td>-</td>
<td>143 (-199)</td>
<td>-96 ± 70</td>
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</table>

Subject #4 did not progress to study LAL-CL04 and is therefore not included in this table. Baseline is defined as date of first dose.

*Subject paused treatment at week 38.

ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase. GGT, gamma glutamyl transpeptidase; LDL, low-density lipoprotein; HDL, high-density lipoprotein.

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Fig. 3. Mean percent change from LAL-CL01 baseline for serum lipids in LAL-CL04 study (n = 8). All one-week-post-infusion laboratory data are presented one week after the week 24 visit. HDL, high-density lipoprotein-cholesterol; LDL, low density lipoprotein-cholesterol; Trig, triglycerides.

Fig. 4. Absolute LDL values over time (n = 8).
epinephrine. The symptoms resolved over the next three hours. Given the nature of this reaction and the absence of similar events in other subjects, treatment was paused in this subject to allow further investigation. Anti-sebelipase (total and IgE) and anti-egg-white protein antibodies were negative. Skin testing results were inconclusive in that a positive skin test at the highest concentration of intradermal sebelipase alfa was seen in both this subject and a concurrently treated study subject who did not experience a hypersensitivity reaction. After an independent safety committee review, the subject was successfully re-started on sebelipase alfa initially at a reduced dose, infusion rate and pre-medication. The subject is now receiving his previous dose (1 mg/kg) at the standard infusion rate. The subject has since received 15 infusions (as of April 24, 2014) with no recurrence of hypersensitivity-like symptoms.

One or more AEs were reported by all subjects during the trial. Events reported by three or more subjects included headache, cold, sore throat, abdominal pain/cramping, nausea, diarrhoea, and back pain. AEs were mainly mild and unrelated to sebelipase alfa. No related serious AEs were reported up to week 52. With the exception of the infusion-related reaction (IRR) event described above for subject #2, there was no requirement for pre-medication or modification of the infusion rate due to infusion-related side effects. In the seven subjects who received treatment up to week 52 and the one who received treatment up to week 38, no anti-drug antibodies were detected.

Discussion

LAL D is a rare, under-diagnosed progressive disease with early mortality and significant morbidity mainly due to liver complications. In the absence of any approved therapies, medical management is largely confined to the use of statins and other lipid-lowering therapies, and liver transplantation for those with decompensated cirrhosis and liver failure. The failure of statins to substantially improve liver manifestations in LAL D patients is clinically important as liver disease progression is clearly documented and appears relatively common in patients on statins who have undergone serial liver biopsies [2].

Sebelipase alfa is well-tolerated, with rapid and consistent decreases in serum transaminases, and improvements in dyslipidemia after an initial early rise in serum lipids (due to lysosomal lipid mobilization) and continues to be well-tolerated with a safety profile largely consistent with previous experience [17]. Most reported AEs were infrequent, mild and unrelated to study drug. The most notable new finding was a possible hypersensitivity-like IRR after 36 weeks of treatment in one subject, but this subject has successfully re-started treatment. Although the number of subjects is small, the frequency of IRRs appears low relative to other approved enzyme replacement therapies [24].

None of the subjects to date in this study have developed detectable antibody responses, and this is consistent with the low frequency of infusion reactions in the overall population. The reasons for the absence of evidence of immunogenicity of sebelipase alfa in this LAL D adult population are unknown. It is possible that the presence of at least one copy of the common mutation (exon 8 splice junction mutation (c.894G>A) may allow production of small amounts of residual enzyme in these adult subjects [25] (Supplementary Table 1) and influence immunogenicity.

Assessment up to 52 weeks provides further evidence that sebelipase alfa produces sustained improvements in markers of liver damage and in the dyslipidemia associated with LAL D. The long-term liver effects at week 52 were consistent with those seen at week 12; the statistically significant mean decreases from baseline in ALT (76 U/L at baseline, decreased by 44 U/L [52%]) and AST (56 U/L at baseline, decreased by 20 U/L [36%]) seen at week 12, were maintained up to week 52 (ALT decreased by 49 U/L [58%), AST decreased by 23 U/L [40%], p = 0.016). Improvements were also seen in other biochemical markers of liver disease including reductions in GGT and alkaline phosphatase. The rapid and sustained improvements in the transaminase profile were accompanied by evidence of reductions in liver lipid content as evidenced by a mean decrease in liver volume (12% at week 52) and mean reduction in hepatic PDFF (55% change from LAL-CL04 baseline to week 52). The concordance of effects on these parameters is expected as treatment with sebelipase alfa directly addresses the root cause of the disease and is consistent with findings from studies in a rat model of LAL D [15]. Given that the lysosomal accumulation of CE and/or TG appears to be a potent inducer of liver fibrosis with evidence of fibrosis and cirrhosis within months of birth in the most severely affected patients [12–14], the normalization of transaminases and other hepatic disease markers, and reduction in hepatic PDFF seen in this study suggest that sebelipase alfa may have the potential of reducing the risk of fibrosis and progression to cirrhosis.

In addition to the improvements in liver biochemical parameters with enzyme replacement, all subjects showed improvements in their lipid profile through week 52. These effects on lipid profile were maintained from week 12 onwards, with evidence of additional time-dependent improvements in LDL and HDL through to week 52.

Sebelipase alfa produced a marked reduction in the MR-estimated hepatic PDFF, which suggests that this method can be used to non-invasively quantify the effects of enzyme replacement and other interventions in LAL D patients. Substantial reductions were seen within ten weeks of treatment initiation in LAL-CL04 with further improvements through to week 52. Further support for the effectiveness of sebelipase alfa in mobilization of lysosomal lipid is provided by the marked decrease in the magnitude of the 1-week post-infusion increase in lipids after more than
20 weeks of dosing compared to the effects seen following initiation of treatment in LAL-CL01 and LAL-CL04. Although descriptions of the macroscopic liver appearance in LAL D patients typically describe a striking orange colour, baseline values for hepatic PDFF from study subjects were relatively low compared to values described in NAFLD patients. It is possible that the reported baseline hepatic PDFF measurements in LAL-CL04 are confounded by the prior administration of four doses of sebelipase alfa in these subjects from LAL-CLO1. While this possibility cannot be excluded, relatively low hepatic fat fraction values using conventional methodologies developed for assessing fat content in NAFLD patients, similar to the ones used in this study, have also recently been reported in LAL D patients who have not received prior enzyme replacement [26]. Interestingly, in the Thelwall et al. study [26], LAL D rats and LAL D patients were associated with distinct 1H- and 13C-MRS signatures arising from cholesterol moieties. The high liver cholesterol content relative to TG compared to NAFLD/NASH patients is consistent with previous biochemical analyses [4,27–29]. These findings suggest that fatty liver criteria from radiological methods developed for quantifying hepatic fat in NAFLD may not be optimal for liver diseases where the lipid composition and/or subcellular localization is distinct from NAFLD.

In summary, this long-term follow-up study has shown that the previously reported benefits of enzyme replacement with sebelipase alfa up to 12 weeks of therapy are sustained through week 52 with significant improvements in transaminases, lipid profile, liver volumes and hepatic PDFF in these LAL D patients. Sebelipase alfa appears well-tolerated with a low frequency of AEs and no evidence of anti-drug antibody formation in this study. In contrast to many therapies used in the treatment of more common but less well molecularly defined diseases, sebelipase alfa addresses the root cause of LAL D with favourable effects on a broad range of abnormalities described in the affected patients.

Though key limitations of this study include the small number of patients evaluated and the open-label trial design, these results are encouraging and additional data from the randomized placebo-controlled trial (ARISE: NCT01757184) will elucidate the efficacy and safety of sebelipase alfa in LAL D.

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Conflict of interest

Synageva BioPharma Corp. is the manufacturer of sebelipase alfa and conducted the statistical analysis.

Authors’ contributions

All authors had access to the study data, reviewed early and final drafts of the manuscript, and were fully responsible for the content and all editorial decisions related to this manuscript.

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Supplementary data

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References

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