Genetics in Medicine (2022) 24, 1425–1436

ELSEVIER

Genetics in Medicine

www.journals.elsevier.com/genetics-in-medicine

CLINICAL TRIAL

A randomized, placebo-controlled clinical trial evaluating olipudase alfa enzyme replacement therapy for chronic acid sphingomyelinase deficiency (ASMD) in adults: One-year results

ARTICLE INFO

Article history:
Received 10 January 2022
Received in revised form
23 March 2022
Accepted 28 March 2022
Available online 26 April 2022

Keywords:
Diffusing capacity of the lung for carbon monoxide
Niemann-Pick type B
Niemann-Pick type A/B
Organomegaly
Recombinant human acid sphingomyelinase

ABSTRACT

Purpose: This trial aimed to assess the efficacy and safety of olipudase alfa enzyme replacement therapy for non–central nervous system manifestations of acid sphingomyelinase deficiency (ASMD) in adults.

Methods: A phase 2/3, 52 week, international, double-blind, placebo-controlled trial (ASCEND; NCT02004691/EudraCT 2015-000371-26) enrolled 36 adults with ASMD randomized 1:1 to receive olipudase alfa or placebo intravenously every 2 weeks with intrapatient dose escalation to 3 mg/kg. Primary efficacy endpoints were percent change from baseline to week 52 in percent predicted diffusing capacity of the lung for carbon monoxide and spleen volume (combined with splenomegaly-related score in the United States). Other outcomes included liver volume/function/sphingomyelin content, pulmonary imaging/function, platelet levels, lipid profiles, and pharmacodynamics.

Results: Least square mean percent change from baseline to week 52 favored olipudase alfa over placebo for percent predicted diffusing capacity of the lung for carbon monoxide (22% vs 3.0% increases, $P = .0004$), spleen volume (39% decrease vs 0.5% increase, $P < .0001$), and liver volume (28% vs 1.5% decreases, $P < .0001$). Splenomegaly-related score decreased in both groups ($P = .64$). Other clinical outcomes improved in the olipudase alfa group compared with the placebo group. There were no treatment-related serious adverse events or adverse event–related discontinuations. Most adverse events were mild.

Conclusion: Olipudase alfa was well tolerated and associated with significant and comprehensive improvements in disease pathology and clinically relevant endpoints compared with placebo in adults with ASMD.

Introduction

Olipudase alfa is a recombinant human acid sphingomyelinase (ASM) in development to address non–central nervous system manifestations of ASM deficiency (ASMD), an autosomal recessive lysosomal storage disease caused by pathogenic variants in the *SMPD1* (EC3.1.4.12) gene encoding ASM.[1] ASMD (historically known as Niemann-Pick disease [NPD] types A and B[1]) is associated with a spectrum of disease phenotypes. ASMD type B (OMIM

*Correspondence and requests for materials should be addressed to Melissa Wasserstein, Chief, Division of Pediatric Genetic Medicine, Professor of Pediatrics and Genetics, The Children's Hospital at Montefiore, The University Hospital for Albert Einstein College of Medicine, 3411 Wayne Ave, 9th Floor, Bronx, NY 10467. *E-mail address:* mwassers@montefiore.org

A full list of authors and affiliations appears at the end of the paper.

doi: https://doi.org/10.1016/j.gim.2022.03.021

607616; chronic visceral ASMD, NPD type B) and ASMD type A/B (chronic neurovisceral ASMD, NPD type A/B, or intermediate phenotype) have disease onset from childhood to early adulthood. ASMD type B has little or no central nervous system involvement, whereas ASMD type A/B has less severe neurologic manifestations than those observed in ASMD type A, which is uniformly fatal in early childhood.[2-4] Visceral manifestations across ASMD subtypes include interstitial lung disease (ILD)[2,5-8] with decreased diffusing capacity of the lung assessed using carbon monoxide (DL_{CO}),[5,8-10] hepatosplenomegaly, progressive liver disease with cirrhosis and fibrosis,[11] dyslipidemia, osteopenia, and thrombocytopenia.[2,5-8,12] Advanced lung disease/lung infections, liver failure, and bleeding are leading causes of early mortality in adults with ASMD.[13]

In open-label clinical trials in adults and children,[14-16] olipudase alfa decreased sphingomyelin storage, reduced organomegaly, and improved lung and liver function and other nonneurological parameters of chronic ASMD. This report presents results of the placebo-controlled trial of olipudase alfa in adults with ASMD.

Materials and Methods

Study design and participants

This phase 2/3 randomized, placebo-controlled, double-blind study in adults with ASMD (ASCEND/NCT02004691/EudraCT 2015-000371-26) was conducted in 13 countries between December 18, 2015 and October 17, 2019 (52-week primary analysis period). Institutional Review Boards approved the protocol, and patients provided written informed consent. Adults diagnosed with ASMD by enzymatic assay and/or genotyping with DL_{CO} ≤70% of predicted normal value, spleen volume ≥6 multiples of normal (MN), and splenomegaly-related score (SRS) ≥5 were eligible. ASMD types B and A/B were not differentiated. Exclusion criteria included mean platelet counts $<60 \times 10^9$/L, alanine aminotransferase (ALT) or aspartate aminotransferase >250 IU/L, or total bilirubin >1.5 mg/dL at screening (except for patients with Gilbert syndrome).

Olipudase alfa administration

Patients were randomized 1:1 to receive placebo (0.9% saline) or olipudase alfa via intravenous infusion once every 2 weeks. Randomization was performed centrally by an interactive voice response system, which generated the patient randomization list and allocated patient numbers. Patients, investigators, and the sponsor study team were blinded to the identity of study treatment; all patients regardless of treatment assignment underwent dose escalation in the same manner. The dose escalation scheme[14] began at 0.1 mg/kg with scheduled increases every 2 weeks to a final target maintenance dose of 3 mg/kg or maximum tolerated dose. Dosing schedules were adjusted per tolerability and prespecified dose-limiting toxicity criteria (defined in Supplemental Table 2).

Efficacy outcomes

Percentage change from baseline to week 52 in % predicted DL_{CO}[17,18] adjusted for hemoglobin and ambient barometric pressure and spleen volume (in MN[19]) were independent primary efficacy endpoints. The spleen primary efficacy endpoint in the United States was spleen volume combined with SRS.

DL_{CO} was standardized according to American Thoracic Society/European Respiratory Society guidelines.[20] Spleen volume was determined by abdominal magnetic resonance imaging.[19] The proportion of patients who had an absolute change from baseline value in percent predicted DL_{CO} of at least 15% and the proportion of patients who had at least a 30% reduction in spleen volume were prespecified analyses.

Secondary endpoints included liver volume (in MN[19]), platelet counts, and patient-reported outcomes (PROs) (Brief Fatigue Inventory scale, Brief Pain Inventory, Functional Assessment of Chronic Illness Therapy-Dyspnea [FACIT-Dyspnea] symptom scale). SRS is a PRO developed from a subset of assessments used in myelofibrosis[21] trials. The SRS rates 5 items aiming to measure the impact of splenomegaly on patient quality of life (abdominal pain, abdominal discomfort, early satiety, abdominal body image, and ability to bend down) on a scale of 0 (absent) to 10 (worst imaginable). SRS was used for the first time in this clinical trial and has not been validated in patients with ASMD. SRS was a secondary endpoint in all countries except the US.

Additional endpoints were ILD[22] assessment by high-resolution computed tomography (HRCT) and chest X-ray,[16] pulmonary function tests,[20] fasting lipid levels,[23-25] liver enzyme levels, liver sphingomyelin content (quantified from liver biopsies by computer morphometry of high-resolution light microscopy images),[24,25] and PROs (36-Item Short Form Health Survey, EQ-5D-5L, and Niemann Pick Type B Health Assessment Questionnaire).

Metabolite and biochemical marker analyses

Lysosphingomyelin was measured in plasma by liquid chromatography–tandem mass spectrometry.[24] Chitotriosidase activity in plasma was normalized by doubling values for individuals who were heterozygous for the null *CHIT1* allele 24 base pair duplication in exon 10 and excluding values from patients who were homozygous for this variant.

Safety

Safety assessments included physical examinations, cardiac evaluations, clinical laboratory testing, safety biomarker

plasma levels, and reporting of adverse events and infusion-associated reactions (IARs) (typically occurring within 12 to 72 hours after olipudase alfa infusion and indicative of an inflammatory response characterized by symptoms, such as pyrexia, nausea, vomiting, fatigue, and pain, associated with increases in proinflammatory laboratory values, such as high sensitivity C-reactive protein and/or ferritin).

Immunogenicity was assessed using a validated enzyme-linked immunosorbent assay to determine titers for immunoglobulin G antidrug antibody (ADA). Positive samples were evaluated for neutralizing antibodies against enzyme catalytic activity and cellular uptake, using optical density absorbance for catalytic activity and cellular imaging of fluorescent intensity of Niemann-Pick fibroblasts for cellular uptake.

Analyses

Sample size calculations were made so that comparisons between the olipudase alfa and placebo groups for percentage change from baseline to week 52 in spleen volume and percent predicted DL_{CO} had greater than 99% and 93% power, respectively, using a 2-sided t-test at the 5% significance level. To determine significance of the primary endpoints, multiplicity adjustments were based on the Hochberg method. Hypothesis testing of secondary endpoints proceeded using sequential testing at the 5% significance level if statistical significance was reached on both primary endpoints. Formal testing in subsequent steps was stopped if an endpoint was not significant, and *P* values for subsequent endpoints in the sequence were considered exploratory and interpreted at the nominal level. Details of hypothesis testing methods and hierarchy of endpoints are provided in Supplemental Material.

All treated patients were included in safety and efficacy analyses. Demographic and disease characteristics were summarized using descriptive statistics. The percentage change from baseline to week 52 for primary and secondary endpoints was analyzed using the mixed model for repeated measures with baseline values, baseline age, treatment arm, study visit, and study visit × treatment arm interaction as covariates. Comparisons between treatment groups were made using least square (LS) mean contrasts at the week 52 visit. SRS information collected by eDiary over 7 consecutive days was averaged for respondents with data for at least 4 of 7 days.

Results

Patients

Of 62 screened patients, 2 withdrew consent, 24 were screen failures (Figure 1), and 36 were randomized 1:1 to the placebo or olipudase alfa group; 35 patients completed the 52-week primary analysis period. One patient in the placebo group withdrew owing to poor compliance.

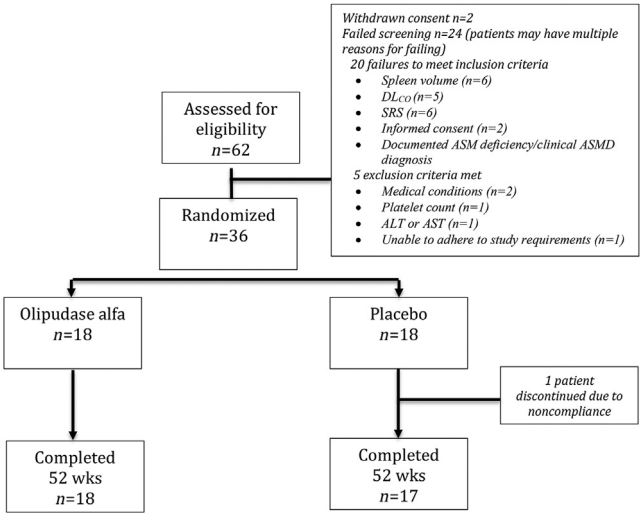

Figure 1 Patient disposition. ALT, alanine aminotransferase; ASM, acid sphingomyelinase; ASMD, acid sphingomyelinase deficiency; AST, aspartate aminotransferase; DL_{CO}, diffusing capacity of the lung for carbon monoxide; SRS, splenomegaly-related score.

Patient characteristics are shown in Table 1. Age ranged from 18.6 to 65.9 years. Most patients (32/36, 89%) self-identified as White and were from the United States, Europe, or South America. The placebo group had proportionally more women than men and a lower age of diagnosis than the olipudase alfa group.

Treatment

Mean compliance was 95% of scheduled infusions in both groups. One patient in the placebo group missed multiple infusions and withdrew, and 1 patient in the olipudase alfa group missed multiple infusions because of adverse events unrelated to the study drug and reached the 0.6 mg/kg dose through week 52 (but subsequently achieved the target dose in the trial extension). Another patient in the olipudase alfa group had a temporary dose reduction during dose escalation for 1 infusion (owing to a non–drug-related adverse event of pharyngitis) before resuming escalation to the target dose.

Primary efficacy analyses

Mean baseline percent predicted DL_{CO} (placebo: 48.5 ± 10.8, olipudase alfa: 49.4 ± 11.0) indicated moderate impairment.[28] The LS mean percentage change from baseline to week 52 was greater in the olipudase alfa group (increase of 21.97%) than in the placebo group (increase of 2.96%) (difference = 19.01, $P = .0004$), and improvement was evident at week 26 (difference = 14.14, nominal $P = .0015$) (Figure 2A1). No olipudase alfa–treated patient had a decrease in DL_{CO} (Figure 2A2); percent change from baseline at week 52 ranged from increases of 7% to 78%. In

Table 1 Demographics and baseline characteristics by treatment group and overall population.

Characteristic	Placebo (n = 18)	Olipudase Alfa (n = 18)	Overall (n = 36)
Age, y			
Mean ± SD	33.5 ± 17.1	36.2 ± 12.7	34.8 ± 14.9
Median (min:max)	24.1 (18.6:65.9)	34.9 (18.8:59.9)	29.9 (18.6:65.9)
Sex, *n* (%)			
Female	13 (72)	9 (50)	22 (61)
Male	5 (28)	9 (50)	14 (39)
Self-identified race, *n* (%)			
Asian	1 (6)	1 (6)	2 (6)
White	16 (89)	16 (89)	32 (89)
Other	1 (6)	1 (6)	2 (6)
Self-identified ethnicity, *n* (%)			
Hispanic or Latino	6 (33)	5 (28)	11 (31)
Not Hispanic or Latino	12 (67)	12 (67)	24 (67)
Not reported	0	1 (6)	1 (3)
Age at ASMD diagnosis, y			
Mean ± SD	14.6 ± 16.1	21.4 ± 20.3	18.0 ± 18.4
Median (min:max)	6.4 (1:49)	16.1 (1:58)	6.4 (1:58)
Years since ASMD diagnosis			
Mean ± SD	18.9 ± 13.7	14.8 ± 13.4	16.8 ± 13.5
Median (min:max)	16.5 (2:51)	12.9 (0:42)	16.3 (0:51)
ASM activity in peripheral leukocytes, nmol/h/mg[a]			
Mean ± SD	0.121 ± 0.086	0.118 ± 0.073	0.119 ± 0.079
Median (min:max)	0.14 (0:0.30)	0.10 (0:0.27)	0.10 (0:0.30)
SMPD1 genotype, *n* (%)			
Homozygous for p.Arg610del	1 (5.6)	4 (22.2)	5 (13.9)
Heterozygous for p.Arg610del	5 (27.8)	5 (27.8)	10 (27.8)
Other variants	12 (66.7)	9 (50.0)	21 (58.3)

Patients were enrolled from Argentina, Australia, Brazil, Chile, France, Germany, Italy, Japan, the Netherlands, Spain, Turkey, the United Kingdom, and the United States.

ASM, acid sphingomyelinase; *ASMD*, acid sphingomyelinase deficiency; *max*, maximum; *min*, minimum.

[a]Determined by the Thomas Jefferson University LSD testing laboratory, Philadelphia, PA.[26] Analytical validity of assay, enzyme activity less than 10% of the normal mean.[27]

comparison, changes in the placebo group ranged from a decrease of 14% to an increase of 29%. In the olipudase alfa group, 6 patients with moderate impairment at baseline had mild impairment at week 52, and 1 patient with severe impairment at baseline had moderate impairment at week 52. Of 18 olipudase alfa–treated patients, 5 had an absolute increase from baseline value in DL_{CO} of at least 15% vs 0 of 18 patients in the placebo group (nominal P = .075). See Supplemental Table 1 for week 26 and week 52 data.

Mean baseline spleen volume in the olipudase alfa (11.7 ± 4.9 MN) and placebo (11.2 ± 3.8 MN) groups reflected moderate to severe splenomegaly[19] (range = 6.1-20.9) (Figure 3A2). The LS mean percentage change from baseline to week 52 was greater in the olipudase alfa group (decrease of 39.4%) than the placebo group (increase of 0.48%) (difference = −39.9%, P < .0001); improvement in the olipudase alfa group was evident at week 26 (nominal P < .0001) (Figure 3A1). All olipudase alfa–treated patients had reductions in spleen volume (Figure 3A2). Among 5 patients with severe splenomegaly at baseline, 4 improved to moderate levels, and 4 of 12 patients with moderate levels improved to mild by week 52 (Figure 3A2). Spleen volumes were essentially unchanged in the placebo group. Among olipudase alfa–treated patients, 94.4% (17/18) had volume decreases >30% vs 0 of 18 patients in the placebo group. One patient in the olipudase alfa group who did not have a >30% reduction in spleen volume missed multiple infusions (unrelated to olipudase alfa) and had an overall decrease in spleen volume of 17% at week 52.

Mean ± SD baseline total SRS for placebo and olipudase alfa groups were 28.1 ± 10.6 and 24.6 ± 11.1, respectively. Mean scores on the 5 individual items of SRS varied from 3.9 to 6.6 on a 0 to 10 response scale. Both groups had reductions in SRS total score at week 52 with a non–statistically significant difference (1.6; P = .64) (Figure 3B and Supplemental Table 1).

Other efficacy analyses

Pulmonary endpoints

Baseline lung imaging mean HRCT scores[16] for ground glass appearance (olipudase alfa group, 0.65; placebo group, 0.53) and ILD (olipudase alfa group, 2.0; placebo group, 2.1) (Supplemental Table 1) reflected mild to moderate lung disease. The LS mean change in HRCT mean ILD score for both lungs from baseline to week 52 was greater in the olipudase alfa group (decrease of 0.36) than the placebo

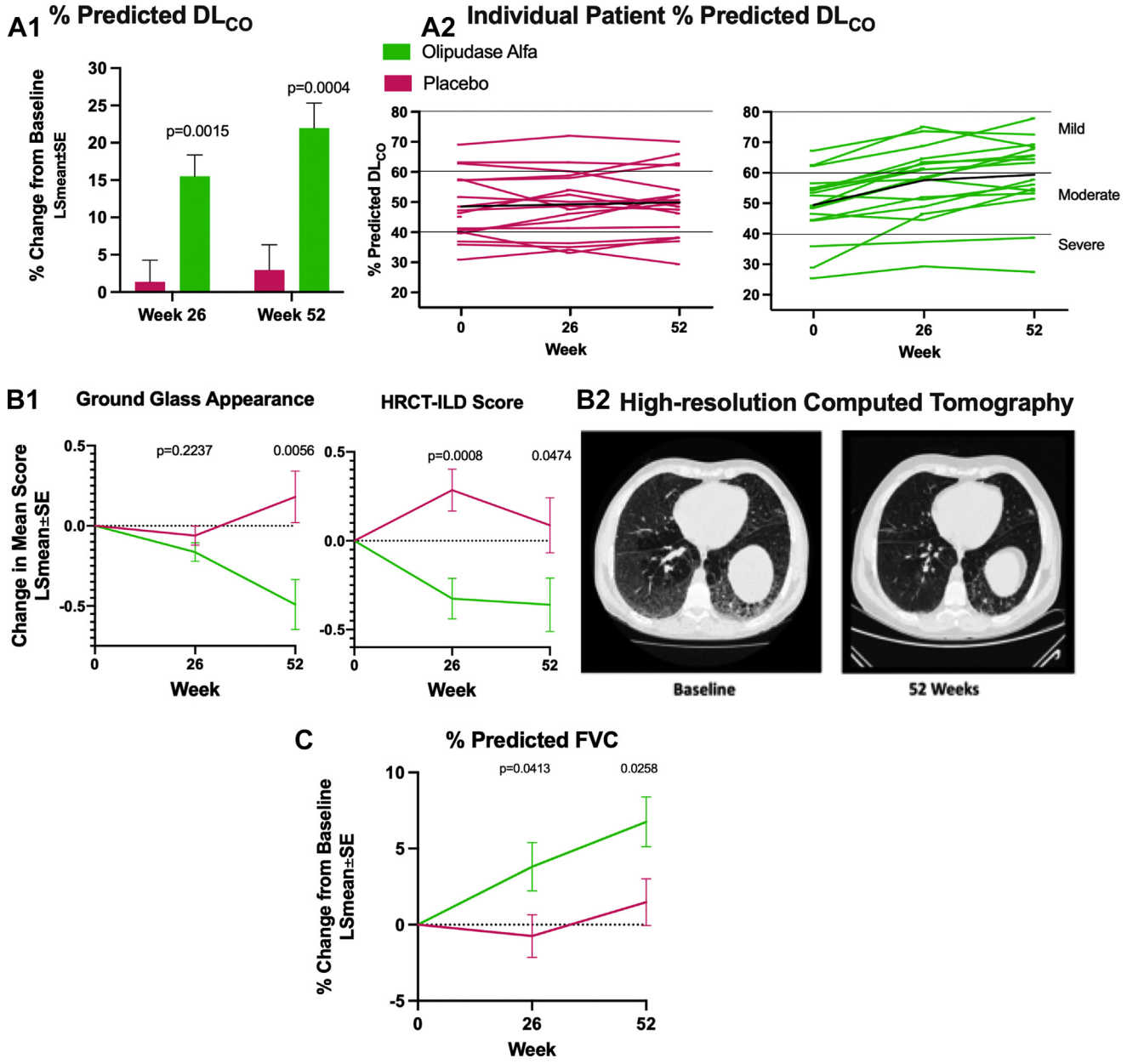

Figure 2 Pulmonary endpoints (diffusing capacity, lung disease imaging, and forced vital capacity) over time in the placebo and olipudase alfa groups. A1. LS mean percent change from baseline in percent predicted DL_{CO} adjusted for hemoglobin and barometric pressure. Analysis of percent change from baseline used a mixed model for repeated measures. A2. Individual patient values for percent predicted DL_{CO} adjusted for hemoglobin and barometric pressure. Percent predicted DL_{CO} values >80% were considered normal/no impairment (LLN), >60% to LLN were considered mild impairment, 40% to 60% were considered moderate impairment, and <40% were considered severe impairment.[28,29] Mean values are indicated by the black lines. B1. Change in mean high-resolution computed tomography scores for ground glass appearance and interstitial lung disease (ILD) scores. Observed means at baseline and subsequent time points are provided in Supplemental Table 1. B2. Illustrative high-resolution computed tomography image of ground glass opacity reflecting sphingomyelin-filled macrophages at baseline and at week 52 for a patient in the olipudase alfa group. Patient had a baseline score for ground glass appearance of 3 (severe, 51% to 100% of lung volume) and a score of 0 at week 52. C. LS mean percent change from baseline in % predicted forced vital capacity (FVC). *P* values are nominal. Observed means ± SD at baseline in the placebo and olipudase alfa groups, respectively, were 83.14% ± 11.75% and 81.62% ± 17.99%. Data for subsequent timepoints are in Supplemental Table 1. DL_{CO}, diffusing capacity of the lung for carbon monoxide; LLN, lower limit of normal; LS, least square.

group (increase of 0.09) (nominal $P = .047$) (Figure 2B1). Results were similar for HRCT ground glass appearance scores (nominal $P = .006$). An illustrative HRCT image from a patient in the olipudase alfa group at baseline and week 52 (Figure 2B2) shows clearance of ground glass opacities (score improved from 3 at baseline to 0 at week 52). Chest X-ray imaging showed improvement in interstitial mean scores after olipudase alfa treatment

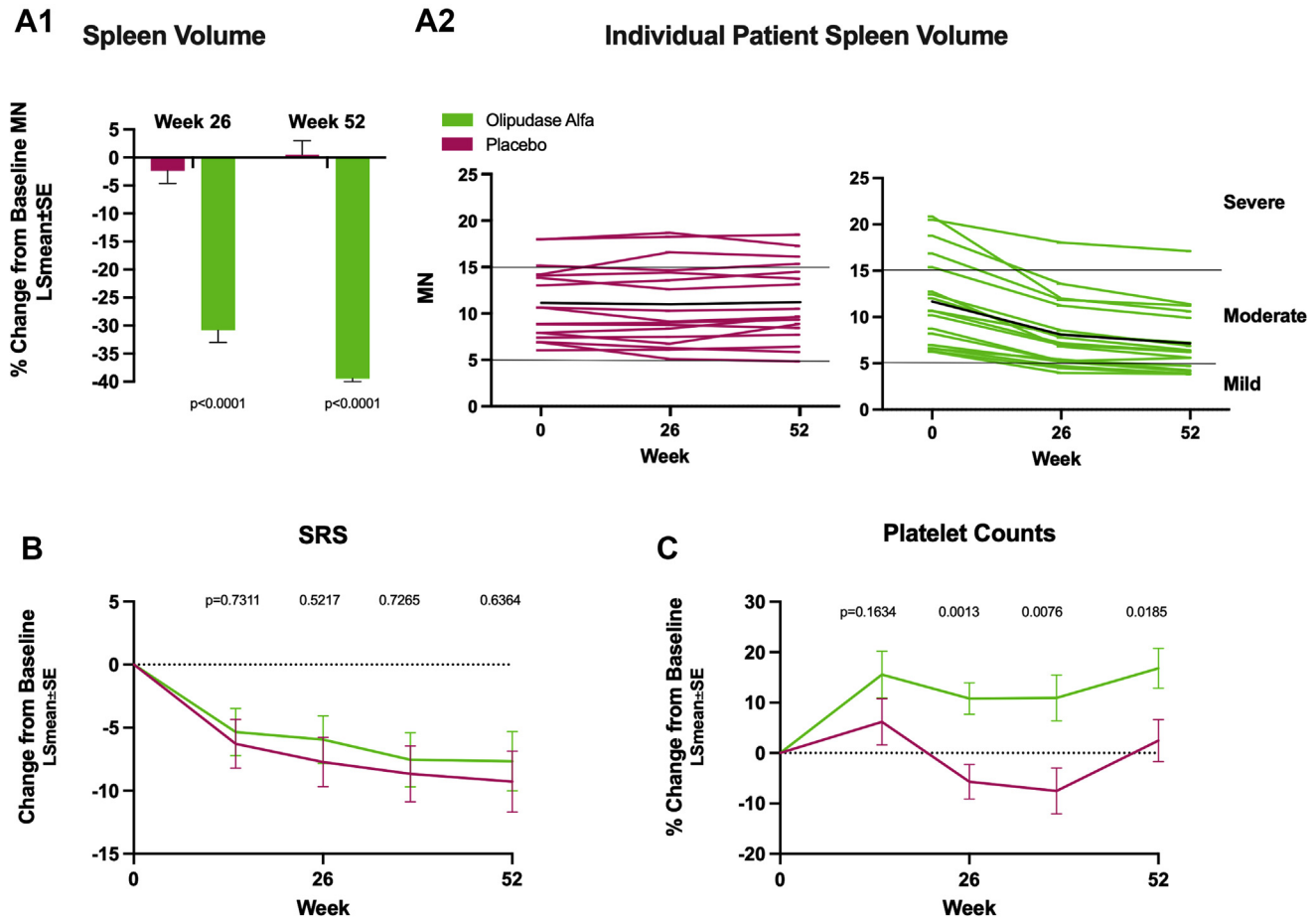

Figure 3 Spleen endpoints and platelet count over time in the placebo and olipudase alfa groups. A1. LS mean percent change from baseline in spleen volume (in multiples of normal [MN]) at week 26 and week 52. Analysis of percent change from baseline used a mixed model for repeated measures. A2. Spleen volume (in MN) for individual patients at baseline, week 26, and week 52. Severe, moderate, and mild splenomegaly are defined as >15, >5 to ≤15, and ≤5 MN, respectively.[19] There were 17 of 18 patients in the olipudase alfa group and 0 of 18 patients in the placebo group with reduction in spleen volume of 30% or more. Mean values are indicated by the solid black lines. B. LS mean change from baseline in SRS. Observed means ± SD at baseline were 28.05 ± 10.56 and 24.55 ± 11.13 for the placebo and olipudase alfa groups, respectively. Data for subsequent timepoints are in Supplemental Table 1. C. LS mean percent change from baseline in preinfusion platelet counts at 14, 26, 38, and 52 weeks. *P* values for percent change from baseline are nominal. Observed mean baseline values in the placebo and olipudase alfa groups, respectively, were 115.6 ± 36.3 and 107.2 ± 0.32 × 10^9/L. Data for subsequent timepoints are in Supplemental Table 1. LS, least square; SRS, splenomegaly-related score.

(−0.91 vs 0.28 in the olipudase alfa and placebo groups, respectively; difference of −1.19, nominal $P < .001$) (Supplemental Table 1).

Volumetric lung function at baseline indicated mild impairment (Supplemental Table 1). Mean percent predicted forced vital capacity was 81.6% and 83.1% in the olipudase alfa and placebo groups, respectively. Mean percent predicted forced expiratory volume in the first second and total lung capacity at baseline were 75.3% and 79.9% in the olipudase alfa group and 78.6% and 77.9% in the placebo group, respectively. The LS mean percentage change in percent predicted forced vital capacity from baseline to week 52 (Figure 2C) showed greater improvement in the olipudase alfa group (6.8%) compared with the placebo group (1.5%) (difference = 5.3%, nominal $P = .026$). Results were similar for percentage change in percent predicted forced expiratory volume in the first second and total lung capacity from baseline to week 52 (nominal *P* values > .05).

Thrombocytopenia

Mean ± SD baseline platelet counts were 115.6 ± 36.3 and 107.2 ± 26.9 × 10^9/L in the placebo and olipudase alfa groups, respectively (range = 63.6-207), reflecting mild thrombocytopenia (week 26 and week 52 means in Supplemental Table 1). The difference in LS mean (SE) percentage change from baseline to week 52 of 14.3 (5.8) favored olipudase alfa ($P = .0185$) (Figure 3C).

Hepatomegaly

Mean ± SD liver volume at baseline indicated moderate hepatomegaly[19] in both the olipudase alfa (1.4 ± 0.3 MN) and placebo (1.6 ± 0.5 MN) groups. The LS mean

percentage change in liver volume from baseline to week 52 (Figure 4A1) showed greater reduction in the olipudase alfa group (decrease of 28.1%) than the placebo group (decrease of 1.5%) (difference = −26.6, $P < .0001$), with improvement observed at week 26 (nominal $P < .0001$). All patients in the olipudase alfa group had liver volume decreases, and 12 patients (67%) with moderate hepatomegaly at baseline improved to mild (Figure 4A2). Liver volumes were largely unchanged in the placebo group.

Sphingomyelin accumulation in liver

Computer morphometry of high-resolution light microscopy images showed mean percent tissue area occupied by sphingomyelin (Figure 4B, bar graph) at baseline to be 28.5% and 30.5% in the olipudase alfa and placebo groups, respectively. At week 52, sphingomyelin burden (mean ± SD percent change from baseline) decreased by 92.7% ± 5.8% in the olipudase alfa group vs an increase of 10.9% ± 42.2% in the placebo group (representative patient images shown in Figure 4B).

Liver function

Mean transaminase baseline levels were elevated relative to normal ranges consistent with ASMD hepatic involvement (Supplementary Table 1). The LS mean percentage change in ALT from baseline to week 52 was −36.6% in the olipudase alfa group and −0.98% in the placebo group (nominal $P = .006$) (Figure 4C1), and reduction was observed at week 14. Results were similar for aspartate aminotransferase and total bilirubin (Supplemental Table 1).

Lipid profiles

At baseline, 33% of patients in the placebo group and 28% of patients in the olipudase alfa group were receiving lipid-lowering agents. Mean baseline levels for proatherogenic lipids (non–high-density lipoprotein cholesterol, triglycerides, apolipoprotein B) and antiatherogenic lipids (high-density lipoprotein cholesterol, apolipoprotein A1) were above and below normal limits, respectively (Supplemental Table 1). Olipudase alfa–treated patients had a greater mean percent reduction from baseline to week 52 for proatherogenic lipid parameters and higher percent increase in antiatherogenic lipid parameters than patients in the placebo group (nominal P values $< .001$ for all) (Figure 4C2; Supplemental Table 1). Differences were observed as early as week 14 depending on parameter.

Metabolite and biochemical marker levels

The sphingomyelin metabolite lysosphingomyelin was elevated at baseline in all patients (range = 157-830 μg/L; upper limit of normal [ULN] = 9.99 μg/L). Preinfusion levels steadily decreased in the olipudase alfa group beginning at the start of treatment, whereas levels remained unchanged in the placebo group (78% decrease in the olipudase alfa group vs 6.1% decrease in the placebo group at week 52) (Supplemental Figure 1A). All patients in the olipudase alfa group except 1 had decreased levels below 200 μg/L by week 52 (mean ± SD = 88.5 ± 101.3; range = 38.2-483 μg/L). One patient in the olipudase alfa group had a level >400 μg/L at week 52 and missed multiple infusions (unrelated to olipudase alfa) (individual patient responses, Supplemental Figure 1A).

Mean plasma levels of the biochemical marker chitotriosidase were elevated at baseline with ranges of 128 to 8610 μmol/L/hr and 262 to 2260 μmol/L/hr in the placebo and olipudase alfa groups, respectively (ULN = 71 μmol/L/hr). Overall, 23 of 36 patients had 2 functional alleles, 8 were heterozygous, and 5 had 2 nonfunctional alleles. Ranges at week 52 were 164 to 7170 μmol/L/hr and 83 to 1190 μmol/L/hr in the placebo and olipudase alfa groups, respectively. The week 52 LS mean percentage change from baseline in chitotriosidase after normalization based on chitotriosidase genotype was a decrease of 54.7% in the olipudase alfa group ($n = 16$) vs a decrease of 12.3% in the placebo group ($n = 15$) (nominal $P = .0003$) (Supplemental Figure 1B).

Patient-reported outcomes

Baseline scores for Brief Fatigue Inventory, Brief Pain Inventory, 36-Item Short Form Health Survey, and EQ-5D-5L reflected moderate impairment, and scores improved in both groups. Changes from baseline were not different between the olipudase alfa and placebo groups for any instrument (data not shown). FACIT-Dyspnea symptom scale scores were largely unrelated to predicted DL_{CO} at baseline and were not significantly associated with the patient's global evaluation of shortness of breath; changes from baseline did not differ between groups (data not shown).

Treatment-emergent adverse events and other safety assessments

Treatment-emergent adverse event profiles are summarized in Supplemental Table 2. All patients experienced at least 1 adverse event, and numbers were similar in the olipudase alfa (242) and placebo (267) groups. The most reported events were headache, nasopharyngitis, arthralgia, upper respiratory tract infection, and cough (Supplemental Table 2). Most events were mild (190/242 [79%] and 206/270 [76%] among olipudase alfa– and placebo-treated patients, respectively). No event led to permanent treatment discontinuation or study withdrawal, and none that were serious were considered potentially related to the study drug. The frequency of clinically significant abnormalities in laboratory findings, vital signs, electrocardiograms, or echocardiograms was similar between treatment groups.

The proportion of patients with adverse events considered potentially related to treatment was greater among olipudase alfa–treated patients (12/18, 66.7%) compared with placebo-treated patients (6/18, 33.3%). Events are listed in Supplemental Table 2, and many overlap with those considered IARs. One olipudase alfa–treated patient with 1 related adverse event of a non–clinically significant increase

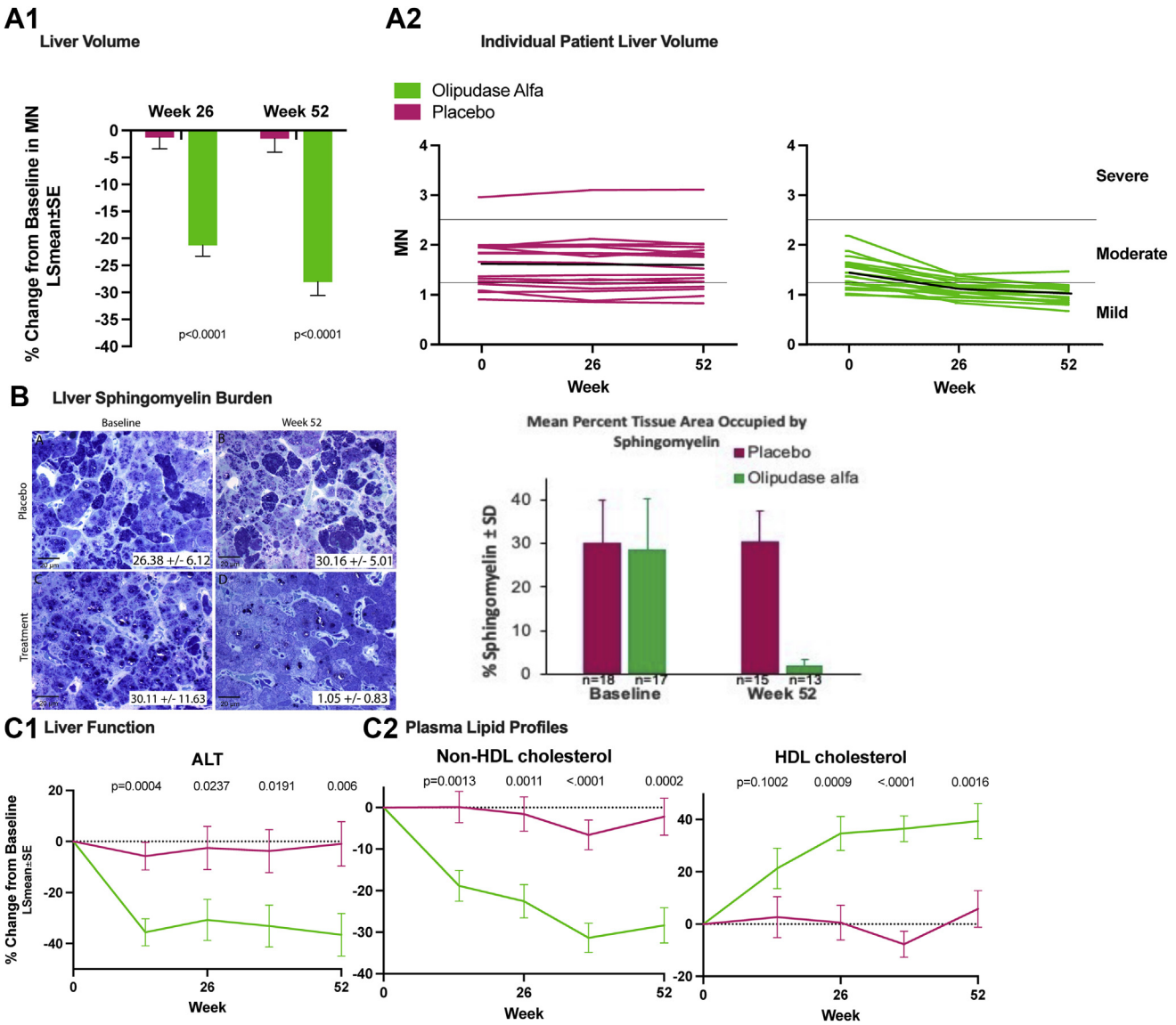

Figure 4 Liver endpoints (liver volume, sphingomyelin burden, and liver transaminase) and lipid profiles over time in the olipudase alfa and placebo groups. A1. LS mean percent change from baseline in liver volume (in multiples of normal [MN]) at week 26 and week 52. Analysis of percent change from baseline used a mixed model for repeated measures. A2. Liver volume (in MN) for individual patients at baseline, week 26, and week 52. Severe, moderate, and mild hepatomegaly were defined as >2.5, >1.25 to ≤2.5, and ≤1.25 MN, respectively.[19] Mean values are indicated by solid black line. B. Liver sphingomyelin burden assessed by computer morphometry of high-resolution light microscopy images (1-µm epoxy resin sections, 600×; bar indicates 20 µm). Images show representative toluidine blue staining of liver biopsies from patients at baseline and after receiving olipudase alfa (treatment) or placebo for 52 weeks. Sphingomyelin appears dark blue. Numbers at lower right corner of each image indicate mean ± SD percentage of tissue area occupied by sphingomyelin. Bar graph shows the mean percent tissue area of sphingomyelin and percent sphingomyelin tissue content for all patients with biopsy data at baseline and week 52. C1. LS mean percent change from baseline in alanine aminotransferase (ALT) levels. *P* values are nominal. Observed mean ± SD baseline values in the placebo and olipudase alfa groups, respectively, were 44.67 ± 30.79 and 40.78 ± 28.32 U/L (0.75 ± 0.51 and 0.68 ± 0.47 µkat/L [multiply by 0.0167 to convert conventional to SI units]). Data for subsequent timepoints are in Supplemental Table 1. C2. LS mean percent change from baseline in proatherogenic non–high-density lipoprotein (non-high density lipoprotein [HDL]) cholesterol. *P* values are nominal. Observed mean ± SD baseline values in the placebo and olipudase alfa groups, respectively, were 5.12 ± 1.75 and 4.43 ± 0.94 mmol/L (197.7 ± 67.6 and 171.0 ± 36.3 mg/dL; multiply by 38.61 to convert SI to conventional units). Optimal level: <3.4 mmol/L, (<130 mg/dL) for individuals at risk of heart disease. Data for subsequent timepoints are in Supplemental Table 1. C3. LS mean percent change from baseline in antiatherogenic HDL cholesterol lipid profiles. *P* values are nominal. Observed mean ± SD baseline values in the placebo and olipudase alfa groups, respectively, were 0.53 ± 0.25 and 0.62 ± 0.22 mmol/L (20.5 ± 9.7 and 23.9 ± 8.5 mg/dL; multiply by 38.61 to convert SI to conventional units). Normal ranges: >0.78 mmol/L, US male; >0.91 mmol/L, US female; >1.2 mmol/L, United Kingdom. Data for subsequent timepoints are in Supplemental Table 1. LS, least square.

in ALT and bilirubin met criteria for a dose-limiting toxicity definition 1. A total of 4 placebo-treated patients experienced 6 events that met dose-limiting toxicity definition 2, criteria and 1 met definition 3 criteria (definitions in Supplemental Table 2).

IARs were more frequent in the olipudase alfa group than the placebo group (8 [44.4%] patients/51 events vs 5 [27.8%] patients/22 events, respectively). The most frequent IAR in the olipudase alfa group was headache. IARs, none of which were categorized as severe or serious, are listed in Supplemental Table 2. IARs considered manifestations of hypersensitivity occurred in 1 patient in the olipudase alfa group (2 events of erythema and urticaria) and 2 placebo-treated patients (2 events of erythema and 1 each of pruritis, erythematous rash, and infusion site urticaria). IARs were managed with supportive medications, including use of premedication per investigator discretion, and/or temporary infusion interruptions. There were no reported cases of acute phase reactions or cytokine release syndrome.

Serious adverse events were reported for 3 olipudase alfa–treated patients (5 events) and 4 placebo-treated patients (11 events); no event was considered treatment-related. One patient in each group had hepatic hemorrhage related to liver biopsy. In the olipudase alfa group, there were single events of cellulitis, viral gastritis, transient ischemic attack, and lower limb fracture. In the placebo group, there were 3 events of epistaxis and single events of liver abscess, appendicitis, peritonitis, anemia, syncope, hemorrhagic shock, and pleural effusion.

Mean preinfusion plasma ceramide levels shown in Supplemental Table 3 were slightly elevated at baseline (~1.5× ULN) relative to the lower limit of normal to ULN range of 1.3 to 3.3 mg/L and gradually decreased in the olipudase alfa group through 52 weeks while remaining unchanged in the placebo group. Mean ± SD percentage change from baseline in the olipudase alfa and placebo groups decreased by 34.8% ± 18.1% and 3.5% ± 27.2, respectively. In the olipudase alfa group, 24- and 48-hour postinfusion ceramide levels were within normal limits by week 12. Ceramide peak responses after infusion were higher after the first infusion despite a minimal dose of olipudase alfa (0.1 mg/kg) compared with responses observed after reaching the maximal dose.

Most patients (16/18 [89%] in the olipudase alfa group and 14/18 [78%] in the placebo group) were negative for ADA at baseline. Four patients (25%) in the olipudase alfa group had treatment-induced ADAs: 2 with transient responses and 2 with persistent responses with peak titers from 50 to 100 (low-titer responses defined as an ADA titer of ≤400). One patient in the placebo group had a transient ADA response. Among 6 patients with positive ADA at baseline (2 in the olipudase alfa group and 4 in the placebo group), none had a treatment-boosted response. No patient had neutralizing antibodies that inhibited cellular uptake of olipudase alfa. Two patients each in the olipudase alfa and placebo groups had transient (1 timepoint each) positive neutralizing antibodies for inhibition of catalytic activity.

Discussion

No disease-modifying treatment for ASMD is currently available, and supportive care is the only management option.[30] The ASCEND clinical trial assessed olipudase alfa vs placebo in adult patients with chronic ASMD. Treatment with olipudase alfa resulted in clearance of sphingomyelin from tissues and produced clinically relevant improvements in multiple aspects of the ASMD clinical profile that are known to persist or worsen over time.[5,7,8,22,23,31] Treated patients showed significant improvement in DL_{CO} and reduction in splenomegaly after 52 weeks of treatment. Improvements in liver volume and increased platelet counts were seen with olipudase alfa compared with placebo. Clinical improvements were consistent with those reported in previous non–placebo-controlled clinical studies of olipudase alfa in adults and children with chronic forms of ASMD.[14-16]

A gradual, stepwise olipudase alfa dose escalation protocol has been used in olipudase alfa clinical trials[14,16] to mitigate rapid production/release of sphingomyelin catabolite(s) thought to contribute to acute phase responses observed in animal studies and the initial phase 1 trial.[32,33] Plasma lysosphingomyelin and ceramide levels gradually decreased in treated patients, signifying debulking of sphingomyelin stores and further supporting olipudase alfa's mechanism of action. Treatment with olipudase alfa resulted in a >90% clearance of sphingomyelin from the liver, whereas sphingomyelin accumulation continued for patients in the placebo group.

Radiographic improvement in ground glass opacities indicated reduction in interstitial cellular infiltrates.[34] ILD worsens with age in patients with chronic ASMD,[2] although some patients may not have overt symptoms.[22,31] The impact of lung involvement on the burden of ASMD is significant, however, respiratory infections and respiratory failure are major contributors to death in adult patients with chronic ASMD.[8,13] Significant improvements in lung diffusing capacity were evident in the olipudase alfa group but not in the placebo group. Improved pulmonary gas exchange was in some cases accompanied by improvements in volumetric lung assessments.

Progressive liver disease, cirrhosis, and liver failure are major contributors to morbidity and mortality in patients with chronic ASMD.[13] Patients treated with olipudase alfa showed significant and sustained improvements in liver disease. Olipudase alfa treatment led to decreases in liver volume, and clearance of sphingomyelin storage in hepatocytes and Kupfer cells was accompanied by improvements in biochemical parameters of liver function. Dyslipidemia, which typically worsens over time in patients with chronic ASMD,[2,8] improved in patients receiving olipudase alfa.

Splenomegaly is a prominent clinical feature of ASMD and contributes significantly to symptoms and disease burden.[10] Olipudase alfa treatment led to a reduction in spleen volume accompanied by increases in platelet counts, reflecting correction of secondary hypersplenism.[35] SRS was used to collect data on symptoms related to

splenomegaly and was adapted for use in the ASCEND trial from a PRO tool used in patients with myeloproliferative diseases.[21] Although myelofibrosis disease pathology is different from ASMD, splenomegaly in both diseases is correlated with increased morbidity and mortality and is a clinically relevant treatment endpoint in clinical trials.[10,21,36] However, the validity, reliability, and sensitivity to change of SRS has not been established in patients with ASMD. SRS improved in both the placebo and olipudase alfa groups without a statistically significant difference between groups, and scores did not correlate with baseline spleen volume. Therefore, SRS does not appear to reflect ASMD disease burden and does not seem to be a useful tool for assessing splenomegaly-related symptoms in this population.

As with SRS, there were no differences between the olipudase alfa and placebo groups in changes from baseline in other quality of life instruments used. Patients reported improvements in shortness of breath, but the FACIT-Dyspnea symptom scale was not significantly associated with these or with percent predicted DL_{CO}. These instruments have been developed and validated in other diseases with pathologies distinct from that of ASMD. In this study, we have not been able to identify PROs that reflect the multisystemic improvements observed after 1 year of olipudase alfa treatment.

Olipudase alfa was well tolerated, with a safety profile consistent with previous studies using within-patient dose escalation.[14-16] Most events were nonserious, non–drug-related, and mild in severity. All IARs were mild to moderate in severity and consistent with events commonly occurring with enzyme replacement therapy. No acute phase reactions or cytokine release syndrome were reported.

Patients with posttreatment ADA had either transient responses or low-tier peak titers. One patient in the placebo group had a transient ADA response, and 6 patients had positive ADA at baseline. The nature of this crossreactivity is not known but has been observed with other biotherapeutics.[37] There were no instances in which neutralizing antibodies inhibited olipudase alfa cellular uptake. Two patients in the olipudase alfa group and 2 in the placebo group had transient positive neutralizing antibodies for inhibition of catalytic activity, the clinical relevance of which is not known.

Conclusions

Treatment with olipudase alfa can clear sphingomyelin from organs and reverse multisystemic clinical features of ASMD. Improvements in lung and liver function should in turn lead to decreased disease burden with potential to improve survival for these patients. Treatment with olipudase alfa was well tolerated. Olipudase alfa has the potential to transform the lives of patients with this progressive multiorgan disorder.

Data Availability

Information regarding data and materials will be made available individually on request.

Acknowledgments

The authors thank patients, families, and research facility clinical staff. The study was sponsored and funded by Sanofi Genzyme. Medical writing support funded by Sanofi Genzyme, United States was provided by Patrice C. Ferriola (KZE PharmAssociates); critical manuscript review and editing were provided by Lisa Underhill (Sanofi). Sanofi Genzyme was involved in the design of the study, data collection, data analysis, interpretation of data, and collaboration with authors on the writing of the manuscript. All authors had full access to the trial data and are responsible for data accuracy and interpretation of the results. The corresponding author had final responsibility for submission of the manuscript.

Author Information

Conceptualization: B.L.T., M.K., A.Z.; Formal Analysis: B.L.T., Y.C., S.F.; Investigation: M.W., L.A.-K., A.B., R.C.G., R.G., N.B.G., C.H., T.I., R.L., O.L., P.M., E.M., M.S., E.S., M.T., J.V., B.L.T.; Writing-review and editing: M.W., L.A.-K., A.B., R.C.G., R.G., N.B.G., C.H., T.I., R.L., O.L., P.M., E.M., M.S., E.S., M.T., J.V., B.L.T., Y.C., S.F., A.Z., M.K.

Ethics Declaration

Local Institutional Review Boards at all study sites approved the protocol, and patients/parents provided written informed consent before screening. The Institutional Review Board of the submitting author was the Biomedical Research Alliance of New York (Lake Success, NY). All clinical data were de-identified. The study adhered to the principles set out in the Declaration of Helsinki.

Conflict of Interest

M.W.: has received travel reimbursement and consulting fees from Sanofi Genzyme.

A.B.: received honoraria for lectures, advisory boards, meetings, and travel support from Sanofi Genzyme and Takeda Shire.

R.G.: has received honoraria, consulting fees, speaker fees, and travel reimbursement from Sanofi Genzyme.

R.L.: has received consulting fees and travel reimbursement from Sanofi Genzyme.

P.M.: has received consulting fees, speaker fees, and travel reimbursement from Sanofi Genzyme.

E.M.: has received consulting fees and honoraria from Sanofi Genzyme.

Employees of Sanofi Genzyme (or were at the time of the study) and own stock in the company: Y.C., S.F., B.L.T., A.Z., M.K.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.03.021) contains supplementary material, which is available to authorized users.

Authors

Melissa Wasserstein[1,*], Robin Lachmann[2], Carla Hollak[3,4], Laila Arash-Kaps[5,6], Antonio Barbato[7], Renata C. Gallagher[8], Roberto Giugliani[9,10,11], Norberto Bernardo Guelbert[12], Takayuki Ikezoe[13], Olivier Lidove[14], Paulina Mabe[15], Eugen Mengel[16], Maurizio Scarpa[17], Eubekir Senates[18], Michel Tchan[19], Jesus Villarrubia[20], Yixin Chen[21], Sandy Furey[21], Beth L. Thurberg[21], Atef Zaher[21], Monica Kumar[21]

Affiliations

[1]Children's Hospital at Montefiore, Albert Einstein College of Medicine, Bronx, NY; [2]Charles Dent Metabolic Unit, National Hospital for Neurology and Neurosurgery, London, United Kingdom; [3]Department of Endocrinology and Metabolism, Amsterdam UMC, University of Amsterdam, Amsterdam, The Netherlands; [4]Amsterdam Gastroenterology Endocrinology Metabolism, Amsterdam, The Netherlands; [5]Villa Metabolica, Department of Pediatric and Adolescent Medicine, University Medical Center Mainz, Mainz, Germany; [6]Clinical Science for LSD, SphinCS, Hochheim, Germany; [7]Department of Clinical Medicine and Surgery, "Federico II" University Hospital, Naples, Italy; [8]Institute for Human Genetics, University of California San Francisco, San Francisco, CA; [9]Medical Genetics Service and DR BRASIL Research Group, Hospital de Clínicas de Porto Alegre (HCPA), Porto Alegre, Brazil; [10]Department of Genetics, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil; [11]National Institute on Population Medical Genetics (INAGEMP), Porto Alegre, Brazil; [12]Metabolic Disease Service Clinica Universitaria Reina Fabiola, Cordoba, Argentina; [13]Department of Hematology, Fukushima Medical University, Fukushima, Japan; [14]Service de Médecine Interne, Diaconesses Croix Saint-Simon Hospital, Paris, France; [15]Servicio de Pediatría, Clínica Santa María, Santiago, Chile; [16]Clinical Science for LSD, SphinCS, Hochheim, Germany; [17]Regional Coordinator Centre for Rare Diseases, University Hospital of Udine, Udine, Italy; [18]Department of Gastroenterology, Istanbul Medeniyet University, Istanbul, Turkey; [19]Department of Genetic Medicine, Westmead Hospital, Sydney, Australia; [20]Hematology Department, Hospital Universitario Ramón y Cajal, Madrid, Spain; [21]Clinical Development, Sanofi, Bridgewater, NJ

References

1. Schuchman EH, Desnick RJ. Types A and B Niemann-Pick disease. *Mol Genet Metab*. 2017;120(1-2):27–33. http://doi.org/10.1016/j.ymgme.2016.12.008.
2. Wasserstein MP, Desnick RJ, Schuchman EH, et al. The natural history of type B Niemann-Pick disease: results from a 10-year longitudinal study. *Pediatrics*. 2004;114(6):e672–e677. http://doi.org/10.1542/peds.2004-0887.
3. Wasserstein MP, Aron A, Brodie SE, Simonaro C, Desnick RJ, McGovern MM. Acid sphingomyelinase deficiency: prevalence and characterization of an intermediate phenotype of Niemann-Pick disease. *J Pediatr*. 2006;149(4):554–559. http://doi.org/10.1016/j.jpeds.2006.06.034.
4. Thurberg BL. Autopsy pathology of infantile neurovisceral ASMD (Niemann-Pick disease type A): clinicopathologic correlations of a case report. *Mol Genet Metab Rep*. 2020;24:100626. http://doi.org/10.1016/j.ymgmr.2020.100626.
5. McGovern MM, Wasserstein MP, Giugliani R, et al. A prospective, cross-sectional survey study of the natural history of Niemann-Pick disease type B. *Pediatrics*. 2008;122(2):e341–e349. http://doi.org/10.1542/peds.2007-3016.
6. McGovern MM, Avetisyan R, Sanson BJ, Lidove O. Disease manifestations and burden of illness in patients with acid sphingomyelinase deficiency (ASMD). *Orphanet J Rare Dis*. 2017;12(1):41. http://doi.org/10.1186/s13023-017-0572-x.
7. McGovern MM, Lippa N, Bagiella E, Schuchman EH, Desnick RJ, Wasserstein MP. Morbidity and mortality in type B Niemann-Pick disease. *Genet Med*. 2013;15(8):618–623. http://doi.org/10.1038/gim.2013.4.
8. McGovern MM, Wasserstein MP, Bembi B, et al. Prospective study of the natural history of chronic acid sphingomyelinase deficiency in children and adults: eleven years of observation. *Orphanet J Rare Dis*. 2021;16(1):212. http://doi.org/10.1186/s13023-021-01842-0.
9. Eskes ECB, Sjouke B, Vaz FM, et al. Biochemical and imaging parameters in acid sphingomyelinase deficiency: potential utility as biomarkers. *Mol Genet Metab*. 2020;130(1):16–26. http://doi.org/10.1016/j.ymgme.2020.02.002.
10. Jones SA, McGovern M, Lidove O, et al. Clinical relevance of endpoints in clinical trials for acid sphingomyelinase deficiency enzyme replacement therapy. *Mol Genet Metab*. 2020;131(1-2):116–123. http://doi.org/10.1016/j.ymgme.2020.06.008.
11. Thurberg BL, Wasserstein MP, Schiano T, et al. Liver and skin histopathology in adults with acid sphingomyelinase deficiency (Niemann-Pick disease type B). *Am J Surg Pathol*. 2012;36(8):1234–1246. http://doi.org/10.1097/PAS.0b013e31825793ff.
12. Garside B, Ho JH, Kwok S, et al. Changes in PCSK 9 and apolipoprotein B100 in Niemann-Pick disease after enzyme replacement therapy with olipudase alfa. *Orphanet J Rare Dis*. 2021;16(1):107. http://doi.org/10.1186/s13023-021-01739-y.
13. Cassiman D, Packman S, Bembi B, et al. Cause of death in patients with chronic visceral and chronic neurovisceral acid sphingomyelinase deficiency (Niemann-Pick disease type B and B variant): literature review and report of new cases. *Mol Genet Metab*.

2016;118(3):206–213. Published correction appears in *Mol Genet Metab*. 2018;125(4):360. https://doi.org/10.1016/j.ymgme.2016.05.001.
14. Wasserstein MP, Jones SA, Soran H, et al. Successful within-patient dose escalation of olipudase alfa in acid sphingomyelinase deficiency. *Mol Genet Metab*. 2015;116(1-2):88–97. http://doi.org/10.1016/j.ymgme.2015.05.013.
15. Wasserstein MP, Diaz GA, Lachmann RH, et al. Olipudase alfa for treatment of acid sphingomyelinase deficiency (ASMD): safety and efficacy in adults treated for 30 months. *J Inherit Metab Dis*. 2018;41(5):829–838. http://doi.org/10.1007/s10545-017-0123-6.
16. Diaz GA, Jones SA, Scarpa M, et al. One-year results of a clinical trial of olipudase alfa enzyme replacement therapy in pediatric patients with acid sphingomyelinase deficiency. *Genet Med*. 2021;23(8):1543–1550. http://doi.org/10.1038/s41436-021-01156-3.
17. Crapo RO, Morris AH. Standardized single breath normal values for carbon monoxide diffusing capacity. *Am Rev Respir Dis*. 1981;123(2):185–189. http://doi.org/10.1164/arrd.1981.123.2.185.
18. Nguyen LP, Harper RW, Louie S. Using and interpreting carbon monoxide diffusing capacity (DLco) correctly. *Consultant*. 2016;56(5):440–445.
19. Pastores GM, Weinreb NJ, Aerts H, et al. Therapeutic goals in the treatment of Gaucher disease. *Semin Hematol*. 2004;41(4)(suppl 5):4–14. http://doi.org/10.1053/j.seminhematol.2004.07.009.
20. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319–338. http://doi.org/10.1183/09031936.05.00034805.
21. Scherber R, Dueck AC, Johansson P, et al. The Myeloproliferative Neoplasm Symptom Assessment Form (MPN-SAF): international prospective validation and reliability trial in 402 patients. *Blood*. 2011;118(2):401–408. http://doi.org/10.1182/blood-2011-01-328955.
22. Mendelson DS, Wasserstein MP, Desnick RJ, et al. Type B Niemann-Pick disease: findings at chest radiography, thin-section CT, and pulmonary function testing. *Radiology*. 2006;238(1):339–345. http://doi.org/10.1148/radiol.2381041696.
23. McGovern MM, Pohl-Worgall T, Deckelbaum RJ, et al. Lipid abnormalities in children with types A and B Niemann Pick disease. *J Pediatr*. 2004;145(1):77–81. http://doi.org/10.1016/j.jpeds.2004.02.048.
24. Thurberg BL, Diaz GA, Lachmann RH, et al. Long-term efficacy of olipudase alfa in adults with acid sphingomyelinase deficiency (ASMD): further clearance of hepatic sphingomyelin is associated with additional improvements in pro- and anti-atherogenic lipid profiles after 42 months of treatment. *Mol Genet Metab*. 2020;131(1-2):245–252. http://doi.org/10.1016/j.ymgme.2020.06.010.
25. Thurberg BL, Wasserstein MP, Jones SA, Schiano TD, Cox GF, Puga AC. Clearance of hepatic sphingomyelin by olipudase alfa is associated with improvement in lipid profiles in acid sphingomyelinase deficiency. *Am J Surg Pathol*. 2016;40(9):1232–1242. http://doi.org/10.1097/PAS.0000000000000659.
26. Wenger D, Williams C. Screening for lysosomal disorders. In: Hommes FA, ed. *Techniques in Diagnostic Human Biochemical Genetics: A Laboratory Manual*. Wiley-Liss; 1991:587–617.
27. Acid sphingomyelinase. Genetic Testing Registry. Accessed February 1, 2022. https://www.ncbi.nlm.nih.gov/gtr/tests/26124/.
28. Pellegrino R, Viegi G, Brusasco V, et al. Interpretative strategies for lung function tests. *Eur Respir J*. 2005;26(5):948–968. http://doi.org/10.1183/09031936.05.00035205.
29. Modi P, Cascella M. Diffusing capacity of the lungs for carbon monoxide. In: *StatPearls*. StatPearls Publishing; 2021. Accessed February 1, 2022. https://www.ncbi.nlm.nih.gov/books/NBK556149/.
30. Wasserstein M, Dionisi-Vici C, Giugliani R, et al. Recommendations for clinical monitoring of patients with acid sphingomyelinase deficiency (ASMD). *Mol Genet Metab*. 2019;126(2):98–105. http://doi.org/10.1016/j.ymgme.2018.11.014.
31. von Ranke FM, Pereira Freitas HM, Mançano AD, et al. Pulmonary involvement in Niemann-Pick disease: a state-of-the-art review. *Lung*. 2016;194(4):511–518. http://doi.org/10.1007/s00408-016-9893-0.
32. Murray JM, Thompson AM, Vitsky A, et al. Nonclinical safety assessment of recombinant human acid sphingomyelinase (rhASM) for the treatment of acid sphingomyelinase deficiency: The utility of animal models of disease in the toxicological evaluation of potential therapeutics. *Mol Genet Metabol*. 2014;2:217–225. http://doi.org/10.1016/j.ymgme.2014.07.005.
33. McGovern MM, Wasserstein MP, Kirmse B, et al. Novel first-dose adverse drug reactions during a phase I trial of olipudase alfa (recombinant human acid sphingomyelinase) in adults with Niemann–Pick disease type B (acid sphingomyelinase deficiency). *Genet Med*. 2016;18:34–40. http://doi.org/10.1038/gim.2015.24.
34. Marten K, Hansell DM. Imaging of macrophage-related lung diseases. *Eur Radiol*. 2005;15(4):727–741. http://doi.org/10.1007/s00330-004-2554-3.
35. Lv Y, Lau WY, Li Y, et al. Hypersplenism: history and current status. *Exp Ther Med*. 2016;12(4):2377–2382. http://doi.org/10.3892/etm.2016.3683.
36. Vannucchi AM, Kantarjian HM, Kiladjian JJ, et al. A pooled analysis of overall survival in COMFORT-I and COMFORT-II, 2 randomized phase III trials of ruxolitinib for the treatment of myelofibrosis. *Haematologica*. 2015;100(9):1139–1145. http://doi.org/10.3324/haematol.2014.119545.
37. Xue L, Clements-Egan A, Amaravadi L, et al. Recommendations for the assessment and management of pre-existing drug-reactive antibodies during biotherapeutic development. *AAPS J*. 2017;19(6):1576–1586. http://doi.org/10.1208/s12248-017-0153-x.