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Original Article

International phase III trial of liprotamase efficacy and safety in pancreatic-insufficient cystic fibrosis patients☆

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Abstract

Background: Most cystic fibrosis (CF) patients have exocrine pancreatic insufficiency (EPI) and need supplementation with pancreatic enzyme replacement therapy (PERT). Liprotamase, a novel non-porcine PERT containing highly purified biotechnology-derived lipase, protease, and amylase, has successfully undergone initial efficacy and safety testing.

Methods: In this international phase III parallel-group, randomized-withdrawal, double-blind placebo-controlled trial, CF patients with EPI 7 years and older, including nutritionally and functionally compromised individuals, underwent baseline testing for coefficients of fat and nitrogen absorption (CFA and CNA) and stool weight and frequency while off PERT. After an open-label treatment period with liprotamase, subjects were randomized 1:1 to one liprotamase or placebo capsule taken with 3 meals and 2 snacks per day. The dose was fixed and increases were not allowed. The same measurements were obtained again after treatment with double-blind study drug or placebo.

Results: 138 subjects were randomized. The adjusted least squares mean (LSM) difference between the treatment and placebo groups for change in CFA was 15.1% ($p=0.001$) for the subgroup with baseline CFA <40%, 8.6% ($p=0.006$) for subjects with baseline CFA ≥40%, and 10.6% ($p<0.001$) for the overall intent-to-treat population. Similar results were seen for change in CNA. Stool weight was significantly decreased although not stool frequency. Liprotamase was well tolerated with no safety concerns identified.

Conclusions: In a CF patient population reflective of that encountered in clinical practice, this trial demonstrated that liprotamase at a fixed dose of one capsule per meal or snack (5 capsules per day) was well tolerated and significantly increased fat absorption as measured by improvement in CFA, significantly increased protein absorption as measured by improvement in CNA, and significantly decreased stool weight.

Keywords: Cystic fibrosis; Pancreatic enzymes; Pancreatic insufficiency; Malabsorption

1. Introduction

Most patients with cystic fibrosis (CF) have exocrine pancreatic insufficiency (EPI) and must use pancreatic enzyme replacement therapy (PERT) to enable them to digest and thus absorb nutrients [1–3]. The currently available PERT formulations, derived by harvesting porcine pancreas glands, require polymeric coating to avoid degradation by gastric acid and

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proteolytic enzymes. Concerns about inconsistent dissolution of the polymeric coating leading to variable absorption and digestion along the length of the small intestine [4], viral contamination [5], batch-to-batch variation and loss of enzyme activity over time, and consequent instances of serious under- and overdosing [2,6–12] led to a requirement for FDA-approved PERTs to have tighter chemistry, manufacturing and controls and to a search for an improved and non-porcine PERT.

Liprotamase is a novel non-porcine PERT, containing a proprietary biotechnology-derived formulation of cross-linked crystalline lipase, crystalline protease, and amorphous amylase that has been designed for purity (no viral contamination) and precise dose standardization. The three enzymes were chosen based upon their broad substrate specificity, resistance against proteolysis, and stability at acid pH for reliable potency of activity in the proximal small intestine [13–16]. Since the stability in harsh environments is engineered into the individual enzymes, polymeric coating is not required.

To date, PERT studies have been small in size, of short duration, and conducted in selective patient populations [17]. Most PERT studies in which CFA is measured have included "dose stabilization" or "normalization" periods during which doses are adjusted based on malabsorption symptoms [17–20]. In some studies, "nonresponders" have been excluded based on the requirement that the on-enzyme CFA must be ≥80% in order for subjects to be eligible for randomization [17,20]. In other studies, the dose selected was thought to maximize fat absorption based on clinical titration [18,19].

The 726 Study Group intended to conduct this phase III trial in a broad-based population of CF patients with PI documented by low baseline CFA or fecal elastase who were in the United States, Europe, South America, and other parts of the world. The responder enrichment strategies outlined above were not employed. An initial phase I study with liprotamase demonstrated good safety and clinical activity in CF patients with EPI [21], and a phase II dose-finding study of 3 different dosages resulted in the selection of a midrange fixed dose at each meal and snack for further study [22]. We report the efficacy and safety results of a placebo-controlled trial of a fixed liprotamase dose of one capsule (containing 32,500 United States Pharmacopeia [USP] U crystallized cross-linked lipase, 25,000 USP U crystallized protease, and 3750 USP U amorphous amylase) with each meal and snack (total of 5 capsules per day), in a broad-based population of pancreatic-insufficient CF patients.

2. Methods

This international phase III parallel-group, randomized-withdrawal, double-blind, placebo-controlled trial was conducted between May 2007 and June 2008 at 23 sites in the United States and 11 sites outside the United States with expertise in treating patients with CF (NCT #449878). The study protocol and informed consent form were approved by the institutional review board/independent ethics committee at each site. All subjects or their legal representatives provided written consent, and in the case of pediatric patients (<18 years of age), assent. Safety oversight was provided and standard stopping rules were set in conjunction with a study-specific committee of the US Cystic Fibrosis Foundation (CFF) Data Safety Monitoring Board.

2.1. Study subjects and design

Males and females ≥7 years of age were eligible for the trial if they had documented evidence of the diagnosis of CF (sweat chloride >60 mmol/L or two CF-causing mutations), had EPI as defined by a fecal elastase [1] ≤100 μg/g stool and CFA ≤80% while off enzymes, were clinically stable, were able to discontinue use of their current PERTs, and were able to undergo the designated testing and inpatient stays. Under the broad-based population study design, no exclusions were made based on BMI, BMI percentile, weight loss, nutritional status, malabsorption symptoms, or PERT dose stability at enrollment. Subjects were excluded if there was a history of fibrosing colonopathy, organ transplantation, significant bowel resection, distal intestinal obstructive syndrome (DIOS) in the prior 6 months, or any acute or chronic diarrheal illness unrelated to EPI. Subjects were also excluded for baseline elevation of liver transaminases >5 times or bilirubin >1.5 times the upper limit of normal, inability to discontinue enteral tube feedings during the study, or known hypersensitivity to food additives.

The study was divided into five phases (Fig. 1), as follows: (1) screening; (2) inpatient off-enzyme baseline phase; (3) open-label outpatient treatment phase of 21 to 31 days during which all subjects received a fixed dose of liprotamase; (4) inpatient randomized double-blind treatment phase of 6 days; (5) second open-label treatment phase of 1 week. Eligible subjects received diet instructions at the screening visit and were admitted to an inpatient facility within the next 4 weeks to begin the off-enzyme baseline phase (phase 2). Each subject ate a high-fat diet, starting 4 days prior to admission, and discontinued the usual PERT 1 day prior to admission. The second day of the wash-out off PERT was performed at the inpatient facility. After baseline blood glucose determination, each subject underwent a starch challenge test (SCT) following a breakfast meal limited to white flour bread (50 g of carbohydrate), followed by serial blood glucose measurements as previously described [22]. The SCT was not performed on subjects receiving treatment with either oral hypoglycemic agents or insulin. After the two PERT-free days, each subject began a controlled 72-hour diet consisting of 2 g of protein per kg per day and 100 g of fat per day (or 38% of total calories per day for children unable to consume 100 g of fat). Two 250-mg FD&C blue #2 marker capsules were given at the beginning and end of the 72 h [21]. Stool was collected after passage of the first blue marker up to and including the second blue marker for determination of CFA, coefficient of nitrogen absorption (CNA), and stool weight and frequency. The collected stool and laboratory serum samples were shipped frozen to Mayo Clinical Laboratory Services (Rochester, MN, USA) for analysis and measurement using NMR spectroscopy [23].

At the completion of the stool collection, subjects were discharged from the inpatient facility and were instructed to take

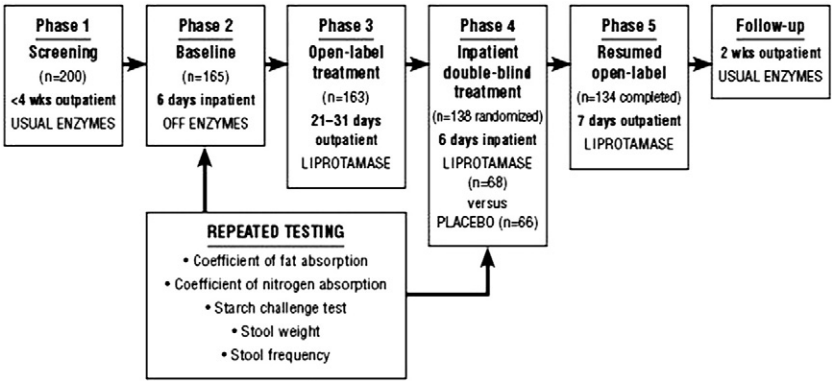

Fig. 1. The international phase III randomized withdrawal, double-blind, placebo-controlled trial of the efficacy and safety of liprotamase in cystic fibrosis (CF) patients with exocrine pancreatic insufficiency (EPI). Trial design and subject disposition.

1 capsule of liprotamase in the middle of each of three meals and two snacks (5 capsules) daily. They were not allowed to adjust this dose based upon symptoms or other considerations. This open-label portion of the study (phase 3) was intended to allow a short adjustment period to liprotamase prior to randomized withdrawal. A CF-specific multivitamin enriched with vitamins A, D, E, and K was provided to all US subjects. Subjects outside the United States were encouraged to take fat-soluble vitamins, but such vitamins were not supplied by the trial sponsor. Subjects with confirmed pancreatic insufficiency defined as a baseline CFA $\leq 80\%$ continued in the open-label liprotamase treatment phase (phase 3) and were eligible for randomization to receive double-blind liprotamase or placebo in the inpatient double-blind treatment phase (phase 4). At randomization, subjects were stratified according to CFA $<40\%$ versus CFA $\geq 40\%$. Subjects with baseline CFA $>80\%$ were not eligible to be randomized. Subjects were also stratified according to whether they were receiving daily acid suppression therapy at baseline; subjects who were receiving daily acid suppression therapy at baseline remained on their prescribed acid suppression drugs and dosages throughout the study. Subjects underwent safety evaluations weekly after beginning the study drug.

The inpatient double-blind treatment phase (phase 4) began 21 to 31 days after the start of open-label treatment for each subject. At that time, subjects were randomized 1:1 either to continue the treatment with liprotamase or to be switched from liprotamase to placebo for a period limited to 6 days. Beginning on day 2 of this randomized study phase, each subject was readmitted to the inpatient facility for repetition of the controlled diet with foods identical to those in the off-enzyme baseline phase and again underwent testing for determination of the same endpoints as in the off-enzyme baseline phase.

Upon discharge, subjects resumed outpatient open-label treatment at the same dose (phase 5) for 1 additional week prior to resuming their usual-care porcine enzyme therapy.

Study drug compliance was assessed throughout the trial by means of pill counts and weekly review of mandatory study drug and vitamin diaries. Safety evaluations were performed throughout the trial, including medical history and physical examinations, vital sign measurements, standard clinical laboratory testing, assessment of adverse events and serious adverse events for severity and causality, and documentation of concomitant medications, treatments, and procedures. After completing the study, each subject returned approximately 2 weeks later for a follow-up safety evaluation.

2.2. Study drug and dosage

Liprotamase is a non-porcine PERT containing highly purified biotechnology-derived enzymes (lipase, protease, and amylase). The lipase and protease are crystallized, and the crystallized lipase is cross-linked to increase shelf life and stability in harsh conditions such as low pH and exposure to proteolytic enzymes. The amylase is amorphous. Selected on the basis of their broad substrate specificity and stability in the pH of the gut and their similarity in activity to their human counterparts, the enzymes do not require polymeric coating for protection against acid hydrolysis or proteolysis. Each lot of the individual drug substances is tested to ensure compliance to specifications, including biologic purity and activity. The three drug substances are blended together with pharmaceutical grade excipients and dispensed into a size 2 gelatin capsule at a fixed ratio of lipase:protease:amylase activities of 1.3:1:0.15. Each liprotamase capsule used in the trial contained 32,500 USP U lipase activity, 25,000 USP U protease activity, and 3750 USP U amylase activity. The rationale for the dosing proportions has been previously described [22]. The single-capsule fixed liprotamase dosing represented the mid-range effective dose identified in the phase II dose-finding study of liprotamase. The placebo consisted of microcrystalline cellulose PH112 dispensed into size 2 gelatin capsules that were identical to the liprotamase capsules in appearance and weight. All subjects were to take one capsule of liprotamase or placebo orally in the middle of each of three meals and two snacks per day during the treatment phases.

2.3. Study endpoints and statistical methods

The primary objective of the study was to determine the efficacy of liprotamase for the treatment of fat malabsorption in

subjects with CF-related EPI. The secondary objectives were to determine the efficacy of liprotamase in these subjects for the treatment of protein malabsorption and carbohydrate malabsorption and to evaluate the ability of liprotamase to decrease stool weight and frequency. The safety objective was to evaluate the safety and tolerability of liprotamase in the study population.

The primary efficacy endpoint was change in CFA between the inpatient off-enzyme baseline phase (phase 2) and the inpatient double-blind treatment phase (phase 4). Secondary efficacy endpoints for similar comparison were change in CNA; response to glucose with the SCT in non-diabetic subjects; and changes in stool weight and frequency.

The primary efficacy analysis was prospectively defined to compare the mean change in CFA for the subjects randomized to liprotamase versus those randomized to placebo in the subgroup with baseline CFA <40% based on the results of our phase 2 study [22] An analysis of covariance (ANCOVA) model with fixed effects for treatment group and acid-suppressant (H-2 blockers, proton pump inhibitors) usage was used to determine the least squares mean (LSM) difference between the treatment groups for the adjusted change in CFA, with corresponding 95% confidence interval (CI) values. Supportive efficacy analyses for the overall population and for the subgroup with baseline CFA ≥40% but ≤80% used the same statistical model with the addition of baseline CFA subgroup for the overall population. An initial test of 2-way interactions was made; interactions significant at $p \leq 0.10$ were to be retained in the ANCOVA model. The secondary endpoint of change in CNA was analyzed using the same approach as that for CFA. Response to glucose was analyzed using a logistic regression model with the same fixed effects as the other analyses, and the relative risk (with associated 95% CI) of not achieving this endpoint was estimated for the liprotamase group versus the placebo group. Changes in stool weight and frequency were analyzed using the ANCOVA models described for change in CFA.

The intent-to-treat (ITT) population was defined as all subjects randomized during the inpatient double-blind treatment phase (phase 4) of the trial. The population for primary safety analyses included all subjects who received at least one dose of study drug and had at least one safety measurement.

The planned sample sizes assumed a treatment to placebo ratio of 1:1, a type I error rate of 0.05, a two-sided test, and a common standard deviation (SD) of 18%. In the overall population, the target sample size of 144 evaluable subjects allowed for the detection of an improvement of 15.56% in mean change in CFA with at least 99% power, which corresponded to an effect size of 0.86, consistent with approved therapies that are considered as moderately effective.

Given a dropout rate of 10% in each baseline CFA subgroup, 46 subjects with baseline CFA <40% and 116 subjects with baseline CFA ≥40% but ≤80% needed to be enrolled. Based on an estimated 8% of the subjects having a baseline CFA >80% [22], approximately 176 total subjects were to be enrolled into the study.

Adverse events, changes in clinical laboratory parameters, and vital signs were analyzed descriptively for each treatment group. Shifts in key laboratory parameters were summarized by treatment group from baseline to the end of the study.

Data management was performed by Quintiles (Livingston, GA, USA), the data management system built with InForm (validated for version 4.5). Study database analyses and reports were conducted and reported using Access 2000, Crystal Reports version 8, and/or SAS version 9.1 (Cary, NC).

3. Results

3.1. Study population

Between May 2007 and June 2008, a total of 200 subjects were screened for study participation (Fig. 1). Of these, 35 did not meet the eligibility criteria, and 2 additional subjects withdrew prior to receiving any study drug; the remaining 163 subjects, constituting the trial safety population, commenced treatment with the study drug during the open-label outpatient treatment phase (phase 3). During the inpatient double-blind treatment phase (phase 4), 138 subjects were randomized at 23 sites in the United States (n=68) and 11 sites outside the United States (n=70: at 3 sites in Slovakia, 3 sites in Poland, 2 sites in Italy, and 1 site each in Argentina, Serbia, and Russia) to placebo or continued treatment with liprotamase. A total of 25 subjects from the safety population were not randomized for the following reasons: baseline CFA >80% (n=14), occurrence of an adverse event (n=4), protocol noncompliance (n=2), withdrawal of consent (n=1), investigator decision (n=1), and other reasons (n=3). Four subjects (2 randomized to liprotamase, 2 randomized to placebo) withdrew after randomization due to the occurrence of an adverse event (n=3) or a protocol deviation (CFA >80%, n=1).

Baseline characteristics for the 138 subjects who were randomized to treatment with liprotamase or placebo are detailed in Table 1. The age distribution was similar to that seen in the 2008 annual data report from the CFF [24], but the subjects in this study had lower baseline nutritional parameters than the US CF registry population. In the ITT population, mean baseline height, weight, and BMI Z-scores were −0.608, −0.697, and −0.517, respectively; of note, subjects outside the United States had considerably lower baseline height, weight, and BMI Z-scores than those of US subjects. In general, the treatment and control groups were well-matched for age, gender, BMI, proportionate size of subgroup with CFA <40%, and use of acid suppressants. However, mean and median FEV_1 values were higher in the placebo group (77.8% and 82.5%, respectively) than in the treatment group (70.2% and 72%, respectively).

3.2. Efficacy

The adjusted mean change in CFA between baseline and double-blind treatment was significantly greater in subjects randomized to liprotamase than in those randomized to placebo in the subgroup with baseline CFA <40% (n=44, 21.2% vs. 6.0%); the LSM difference (favoring the liprotamase vs. placebo subjects) was 15.1% ($p=0.001$). In the subgroup with

Table 1
Baseline characteristics for 138 intent-to-treat (ITT) subjects randomized to liprotamase or placebo.

Baseline characteristics	Liprotamase subjects (n=70)	Placebo subjects (n=68)	All ITT subjects (n=138)
Age (years)			
Mean (SD)	18.5 (7.34)	17.7 (7.42)	18.1 (7.37)
Median (range)	18.0 (7–37)	16.0 (8–44)	17 (7–44)
7 to <12 years	13 (18.6%)	15 (22.1%)	28 (20.3%)
12 to <17 years	16 (22.9%)	20 (29.4%)	36 (26.1%)
≥17 years	41 (58.6%)	33 (48.5%)	74 (53.6%)
Male	45 (64.3%)	40 (58.8%)	85 (61.6%)
Caucasian	69 (98.6%)	65 (95.6%)	134 (97.1%)
US sites	36 (51.4%)	32 (47.1%)	68 (49.3%)
Sites outside US	34 (48.6%)	36 (52.9%)	70 (50.7%)
Weight Z-score [a]			
Mean (SD)	−0.736 (0.862)	−0.658 (0.905)	−0.697 (0.881)
Median (range)	−0.749 (−2.776 to 1.090)	−0.583 (−2.940 to 1.490)	−0.673 (−2.940 to 1.490)
Height Z-score [a]			
Mean (SD)	−0.628 (0.998)	−0.588 (0.992)	−0.608 (0.991)
Median (range)	−0.622 (−3.520 to 1.716)	−0.541 (−2.967 to 1.321)	−0.558 (−3.520 to 1.716)
BMI			
Mean (SD)	19.1 (2.65)	19.2 (3.28)	19.1 (2.96)
Median (range)	19.4 (13.2 to 26.4)	19.4 (13.0 to 28.0)	19.4 (13.0 to 28.0)
BMI Z-score [a]			
Mean (SD)	−0.531 (0.832)	−0.502 (1.015)	−0.517 (0.924)
Median (range)	−0.470 (−2.639–1.129)	−0.442 (−2.962–1.979)	−0.463 (−2.962 to 1.979)
BMI Z-score percentile [a]			
<10th	11 (15.7%)	12 (17.6%)	23 (16.7%)
10th to <30th	21 (30.0%)	17 (25.0%)	38 (27.5%)
30th to <50th	18 (25.7%)	17 (25.0%)	35 (25.4%)
≥50th	20 (28.6%)	22 (32.4%)	42 (30.4%)
FEV_1 % predicted			
Mean (SD)	70.2 (25.15)	77.8 (24.79)	73.9 (25.17)
Median (range)	72.0 (21.0–119.0)	82.5 (29.0–137.0)	79.5 (21.0–137.0)
Baseline CFA			
<40%	24 (34.3%)	20 (29.4%)	44 (31.9%)
≥40% but ≤80%	46 (65.7%)	48 (70.6%)	94 (68.1%)
On acid suppression	27 (38.6%)	26 (38.2%)	53 (38.4%)

SD: standard deviation; BMI: body mass index; CFA: coefficient of fat absorption; FEV_1: forced expiratory volume in 1 second.
[a] Z-scores represent a measure of the normal population that is often used as a reference point (taking into account age and gender) when trying to assess the impact of a therapy on height, weight, and BMI in a study population including subjects who are still growing.

baseline CFA ≥40% but ≤80% (n=94, 7.7% vs. −0.9%), the LSM difference was 8.6% ($p=0.006$), and in the overall ITT population (n=138,13.8% vs 3.2%) the LSM difference was 10.6% ($p<0.001$) (Fig. 2, Table 2). In a post hoc analysis, for alternative baseline CFA cutoff values for which comparison between the treatment groups was possible, the LSM difference in the adjusted mean change in CFA significantly favored liprotamase over placebo (Fig. 3). The lower the baseline CFA, the greater were the CFA differences.

Analysis of the effect of region on the LSM difference in the adjusted mean change in CFA showed differences between treatment sites in the United States and sites outside the United States: a LSM difference of 16.6% ($p<0.001$) for all subjects at the US sites (n=68) versus an LSM difference of 4.2% (p=NS) for all subjects at the sites outside the United States (n=70) (Fig. 2). Sample sizes were too small to allow comparisons within individual countries, although most of the difference in CFA between US sites and sites outside the United States was driven by sites in a single high-enrolling country outside the United States. A careful examination of the study methods and conduct did not reveal systematic errors to account for this finding.

The use of acid suppressants was associated with an enhanced treatment effect of liprotamase in subjects with low baseline CFA (<40%). For this subgroup, the LSM difference (favoring the liprotamase vs placebo subjects) in the adjusted mean change in CFA was 26.6% ($p=0.003$) for those on acid suppressants (overall n=18) compared to 9.6% ($p=0.056$) for those not receiving acid suppressants (overall n=26). For the overall population, the treatment effect of liprotamase compared to placebo was similar in the group receiving acid suppression therapy (overall n=53, LSM difference 12.6%, $p=0.009$) and those not receiving this therapy (overall n=85, LSM difference 11.2%, $p<0.001$) (Fig. 2).

The unadjusted mean change in CNA between baseline and double-blind treatment was significantly greater in subjects randomized to liprotamase than in those randomized to placebo in the subgroup with baseline CFA <40% (21.7% vs 9.0%), in the subgroup with baseline CFA ≥40% but ≤80% (8.1% vs. 1.5%), and in the overall ITT population (13.3% vs. 4.1%)

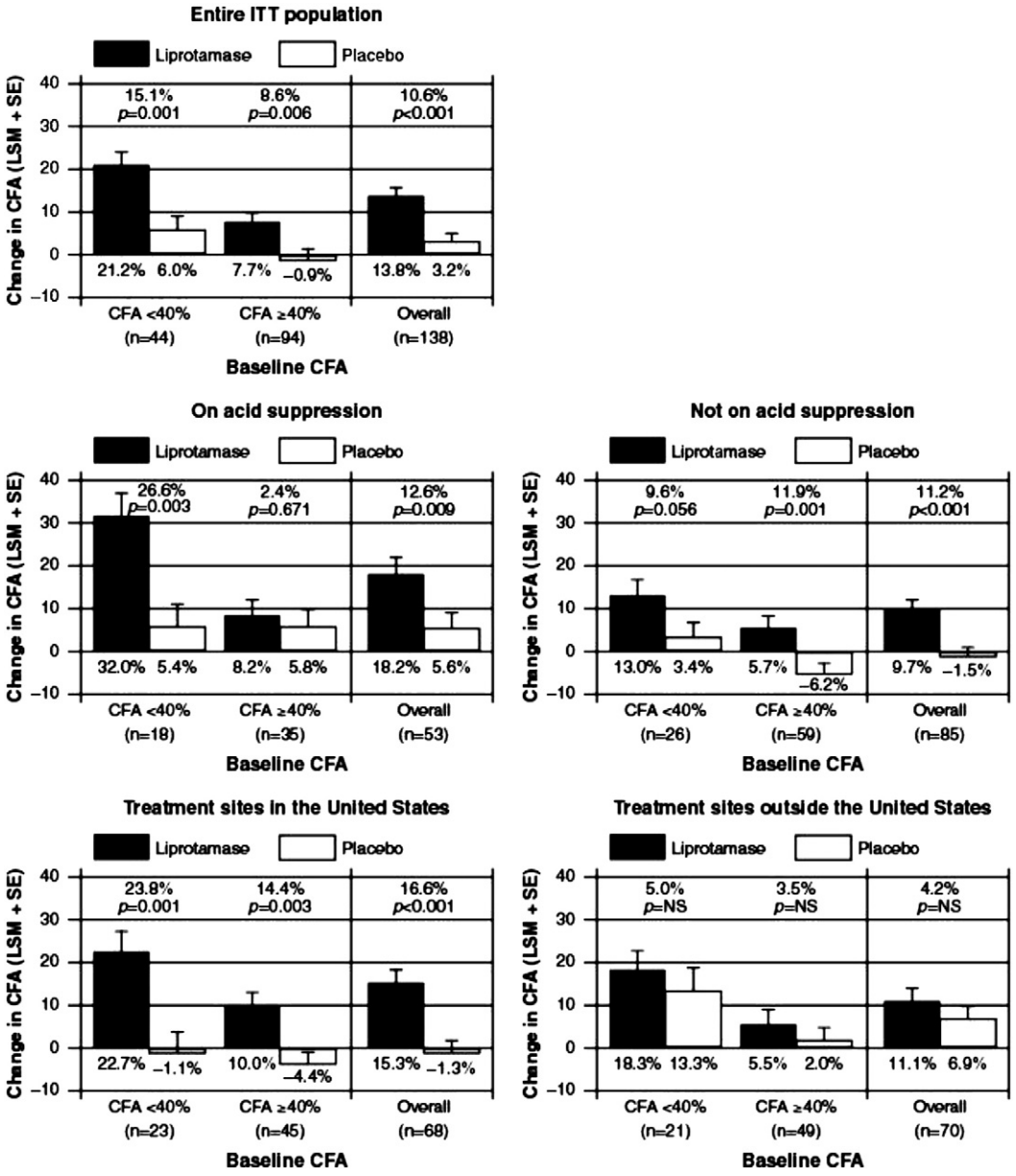

Fig. 2. Mean change from baseline in coefficient of fat absorption (CFA) for CF subjects receiving liprotamase or placebo during the inpatient double-blind treatment phase of the trial, with stratification for baseline CFA, in (A) the entire intent-to-treat (ITT) population, (B) subjects who were continued from baseline on acid suppression versus subjects who did not receive acid suppression, and (C) subjects treated at US sites versus sites outside the United States.

(Table 2). The LSM difference (favoring the liprotamase vs. placebo subjects) in the adjusted mean change in CNA from baseline to double-blind treatment was 12.7% ($p<0.001$) for the subgroup with baseline CFA <40%, 6.6% ($p=0.012$) for the subgroup with baseline CFA ≥40% but ≤80%, and 9.2% ($p<0.001$) for the overall ITT population.

In a pre-specified analysis of the ITT nondiabetic population, 25.0% (13/52) of subjects randomized to liprotamase recorded increases of ≥10 mg/dL in maximum blood glucose with the SCT, compared with 32.7% (17/52) in the placebo group ($p=0.386$).

The adjusted mean change in stool weight between baseline and double-blind treatment was −334.7 g in the ITT liprotamase group overall versus −117.7 g in the ITT placebo group overall. A significant LSM difference of −217.0 g ($p=0.0005$) was found between the liprotamase and placebo groups in terms of change in stool weight.

The mean baseline stool frequency during the marker-to-marker collection was 10.2 in the overall liprotamase group and 9.3 in the overall placebo group. The adjusted mean change from baseline in stool frequency was −2.7 in the liprotamase group versus −1.7 in the placebo group. The LSM difference between the liprotamase and placebo groups in terms of change in stool frequency was −1.0 per 72-hour stool collection ($p=0.0837$). In either case, this stool frequency is clinically acceptable.

Table 2
Change in coefficient of fat absorption (CFA) and coefficient of nitrogen absorption (CNA; in shaded boxes) by baseline CFA in the intent-to-treat (ITT) population.

	Liprotamase subjects (n = 70)	Placebo subjects (n = 68)	LSM difference† (95% CI) ANCOVA *p* value
Baseline CFA < 40% (n)	24	20	15.1% (6.4% – 23.8%)
LSM change in CFA* (SE)	21.2% (2.90%)	6.0% (3.21%)	0.001
Baseline CFA ≥ 40% but ≤80% (n)	46	48	8.6% (2.5% – 14.7%)
LSM change in CFA* (SE)	7.7% (2.23%)	– 0.9% (2.18%)	0.006
Overall ITT population (n)	70	68	10.6% (5.6% – 15.5%)
LSM change in CFA* (SE)	13.8% (1.96%)	3.2% (1.97%)	< 0.001
Baseline CFA < 40% (n)	24	20	12.7% (5.7% – 19.7%)
LSM change in CNA* (SE)	21.7% (2.37%)	9.0% (2.60%)	< 0.001
Baseline CFA ≥ 40% but ≤80% (n)	46	48	6.6% (1.5% – 11.7%)
LSM change in CNA* (SE)	8.1% (1.83%)	1.5% (1.78%)	0.012
Overall ITT population (n)	70	68	9.2% (5.0% – 13.4%)
LSM change in CNA* (SE)	13.3% (1.60%)	4.1% (1.61%)	< 0.001

ANCOVA: analysis of covariance; LSM: least squares mean.
*Analysis conducted using baseline observations carried forward method for subjects with no post-baseline data.
†Change in parameter (CFA/CNA) was analyzed using an ANCOVA model with fixed effects for treatment group and acid suppression usage with adjustment for baseline CFA/CNA. Significant interactions with treatment group were included in the models.
††Change in parameter (CFA/CNA) was analyzed using an ANCOVA model with fixed effects for treatment group, CFA subgroup, and acid suppression usage with adjustment for baseline CFA/CNA. Significant interactions with treatment group were included in the model.

3.3. Safety

During the double-blind period, there were no trends or clinically meaningful differences in adverse events between the liprotamase and placebo groups. Overall, most of the adverse events reported were gastrointestinal or pulmonary in nature and were largely those expected for the underlying CF disease. Eight subjects withdrew from the study because of adverse events, 4 of those for abdominal pain or steatorrhea. Three of these 4 withdrawals occurred prior to randomization. All 12 treatment-emergent serious adverse events reported in this study were deemed to be either not related to the study drug or unlikely to be related. There were 8 pulmonary exacerbations of CF, 1 kidney-stone episode, and 1 pulmonary embolism. One episode of DIOS occurred after 1 open-label-treatment day following the baseline off-enzyme period. One episode of

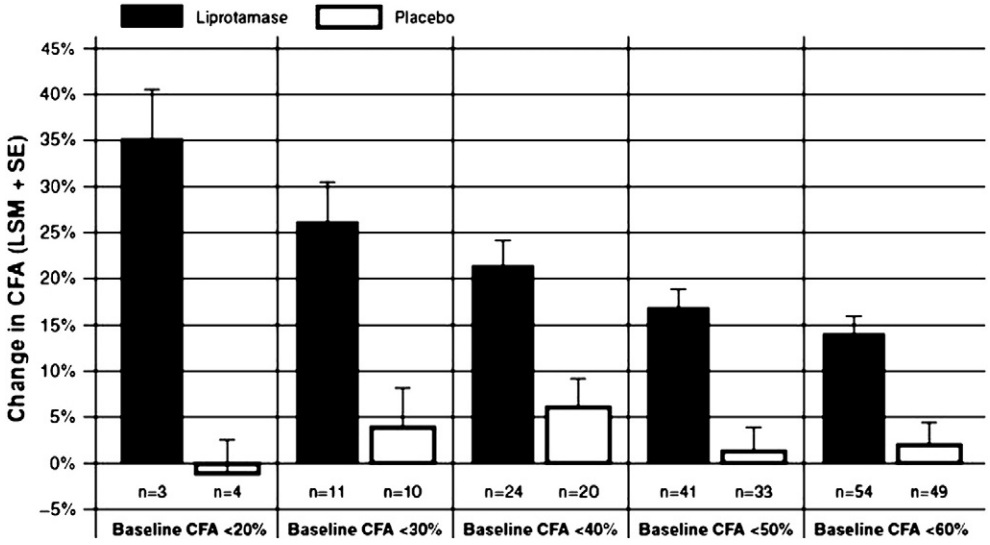

Fig. 3. Adjusted mean change from baseline in coefficient of fat absorption (CFA) stratified by baseline CFA level for CF subjects receiving liprotamase or placebo during the inpatient double-blind treatment phase of the trial. Values are least square means (LSM) plus standard error (SE).

elevated liver transaminases occurred after a fall from a trampoline. No deaths were reported during the study.

There were no major safety concerns identified regarding laboratory values. Abnormal alanine aminotransferase (ALT) values with frequent fluctuations were seen. Nine subjects had ALT levels ≥3 times the upper limit of normal while on study. Five of these 9 subjects did not have normal ALT levels at baseline. All elevations in ALT levels were transient, asymptomatic, and not associated with increases in bilirubin, and all 9 subjects completed the trial.

4. Discussion

This international study, the largest parallel-group, randomized, double-blind, placebo-controlled trial conducted to date in EPI, demonstrated in an "all comer" CF patient population reflective of that encountered in clinical practice that liprotamase at a fixed dose of one capsule per meal or snack (5 capsules per day) significantly increased fat and protein absorption as measured by improvement in CFA and CNA and significantly decreased stool weight. The capsule contents of 32,500 U crystallized cross-linked lipase, 25,000 U crystallized protease, and 3750 U amorphous amylase evaluated in this trial represented the midrange and minimally effective dose from the phase II dose-finding study of liprotamase [22]. This dose was found to be more effective than a dose fivefold lower and as effective as a dose fourfold higher, based upon improvement in CFA and CNA and reduction in stool weight [22]. CFA is an important short-term measure of PERT efficacy, but as with other surrogate markers, it cannot replace a clinically relevant outcome, which, in the case of digestion, would be growth. Although the short-term improvement in CFA achieved with liprotamase in this study, which did not allow for dose increase, was less than what has been seen in other studies comparing PERT to placebo, differences in design, dose, run-in periods, inclusion and exclusion criteria, use of concomitant medications, and methodology for CFA preclude direct comparisons. Subjects in this study who were followed over the course of 1 year showed stable growth on liprotamase.

Weight-based dosing recommendations for PERT were formulated in the early 1990s in response to the epidemic of fibrosing colonopathy, a potential complication when the porcine PERT dosage was "titrated" (increased) on the basis of symptoms with the thought that somehow this would "optimize" the dose [2,12,25]. Although the cause of fibrosing colonopathy is unknown, the enteric coatings [26], altered colonic flora, dietary factors and factors related to bowel motility, activation of fibrogenic cytokines, and the presence of enzymes other than lipase have been implicated [2,12,25]. The current maximum recommended dose of porcine-based PERT is ≤2500 USP U lipase per kg per feeding [2], ≤10,000 USP U lipase per kg per day, or 4000 USP U lipase per gram of fat per day [27]. These empiric recommendations were not based on dose–response studies but rather were set at a level thought to minimize the risk of fibrosing colonopathy. At the available lipase unit strengths for the three currently FDA-approved PERT products, a 50-kg patient taking the target lipase dose studied in available porcine PERT trials could receive 5 or more capsules per meal and snack (25 to 30 or more capsules per day), amounting to 2 to 3 g of enteric coating, ~400,000 USP U lipase, ~1,250,000 USP U protease, and ~2,000,000 USP U amylase per day. In contrast, liprotamase contains no enteric coating (removing the risk of phthalates) and delivers 162,500 USP U crystallized cross-linked lipase, 125,000 USP U crystallized protease, and 18,750 USP U amorphous amylase per day at the dose studied here. Our use of a dosing paradigm with a fixed dose of liprotamase was driven partly by the importance of conveying to patients and care providers that dose should not be continuously titrated up.

Most PERT studies in which CFA is measured have selected a dose intended to maximize fat absorption or included "dose stabilization" or "normalization" periods during which doses are adjusted purportedly to normalize malabsorption symptoms [17–20]. In some studies, "nonresponders" have been excluded based on the requirement that following dose "titration," the on-enzyme CFA must be ≥80% or subjects are not eligible for randomization [17,20]. In contrast, our rigorously controlled parallel-group, randomized-withdrawal trial was open to nutritionally and functionally compromised patients, excluding from randomization only those subjects whose baseline off enzyme CFA was >80%, and the dosage of the study drug was kept fixed. The range of CFA is quite wide in healthy ambulatory CF subjects [28]. In the current trial, as in the previous phase II dose-finding trial of liprotamase [22], the CFA measurements during both the inpatient off-enzyme baseline phase and the inpatient double-blind treatment phase were lower than have been reported in recent trials of porcine-based PERT [18,19]. As noted, substantial protocol design differences make it impossible to compare CFA results across trials. The subgroup stratification and primary efficacy analysis in this phase III trial of liprotamase were based on a post hoc analysis of data from our phase II study, which demonstrated that subjects with baseline CFA ≤40% had a more profound increase in CFA compared with subjects with baseline CFA >40% (31% vs. 8%, $p<0.0001$) [22]. The finding of subjects with the worst baseline fat and protein malabsorption having the greatest improvements when treated had never previously been reported, and was confirmed in the current trial as well as in newer studies of porcine PERTs [18,19].

Some studies have excluded subjects on acid-suppressing medications. In clinical practice, acid suppression therapy is used because of the absence of bicarbonate secretion in EPI. In some reports it has been associated with improved fat absorption, postulated to be independent of digestion and related to improved solubility of bile salts and enhanced micelle formation and function [29,30]. We sought to study a broad-based population, thus we stratified subjects according to whether they were receiving daily acid suppression therapy at baseline prior to randomization. Subjects in this study were classified as on acid suppression therapy if they were taking a proton pump inhibitor and/or an H2 blocker. For the overall population, the treatment effect of liprotamase compared to placebo was similar in the group receiving acid suppression therapy (overall n=53, LSM difference 12.6%) and those not receiving this therapy (overall n=85, LSM difference 11.2%).

There was variability when the CFA data were assessed for United States of America (USA) treatment sites versus those outside the USA. Subjects enrolled outside the USA had lower key baseline nutritional parameters than those in the USA and much lower acid suppressant use (data not shown), suggesting that there are substantial differences in patient management in the USA versus the other countries. Differences in uncontrolled ambulatory diet, overall health status, subject selection, experience in performing PERT studies, and underlying genetic modifiers may also have contributed to outcome variations. There was also variability in response by country, although analysis is limited by small sample sizes. However, although there were centers in six countries besides the United States participating in the study, most of the difference in CFA between USA sites and sites outside the USA was driven by sites in a single high-enrolling country outside the USA. Because the study was not stratified by region or country outside the USA, results for some countries that differ from overall results may be a chance finding due to small numbers of subjects in those countries.

No safety signals emerged during this study. We found notable elevations of ALT in nine subjects, five of whom did not have normal values at baseline. None met the Hy's Law definition of hepatotoxicity [30], thus it is more likely that these elevations were part of the underlying variation in transaminases seen in patients with CF in research studies [31] rather than as a direct result of liprotamase.

In summary, liprotamase significantly improved fat and nitrogen absorption and reduced stool weight at a fixed dose of one capsule (32,500 USP U lipase, 25,000 USP U protease, and 3750 USP U amylase) per meal or snack. No safety concerns were identified. These efficacy and safety data support the use of liprotamase for the treatment of EPI in patients with CF.

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Appendix A. Liprotamase 726 Study Group (CTSA number, if applicable)

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