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Original Article

Icosabutate for the treatment of very high triglycerides: A placebo-controlled, randomized, double-blind, 12-week clinical trial

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KEYWORDS:
Hypertriglyceridemia;
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Triglycerides;
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Remnants

BACKGROUND: Icosabutate is a structurally enhanced omega-3 fatty acid molecule developed with the aim of achieving improved triglyceride (TG)-lowering efficacy, increased potency, and preserved safety compared with conventional prescription omega-3 fatty acid.

OBJECTIVE: To evaluate the efficacy and safety of icosabutate 600 mg once daily in patients with very high TGs.

METHODS: After a 6-8 week run-in period, men and women with TG levels ≥500 mg/dL and ≤1500 mg/dL were randomized to double-blind treatment with placebo or icosabutate 600 mg for 12 weeks. The primary end point was % change from baseline in TGs at 12 weeks.

RESULTS: A total of 87 subjects were randomized. At baseline, median TG (interquartile range) levels were 611 (543–878) and 688 (596–892) mg/dL, and the median change after 12 weeks of treatment was −51% and −17%, respectively, for a placebo-corrected change of −33% ($P < .001$). Adjusted for placebo, icosabutate significantly reduced very low–density lipoprotein cholesterol (−36%, $P < .001$), remnant lipoprotein cholesterol (−34%, $P < .001$), apolipoprotein (Apo) C-III (−35%, $P < .001$), trended toward reduced non–high-density lipoprotein cholesterol (−7%, $P = .064$); significantly increased high-density lipoprotein cholesterol (18%, $P < .001$) and low-density lipoprotein cholesterol (28%, $P < .001$), with a trend of an increased lipoprotein (a; 10%, $P = .054$). No changes were observed in total cholesterol, apolipoprotein B, or apolipoprotein A1. Fasting plasma glucose was unchanged, whereas fasting plasma insulin was reduced ($P = .001$) with icosabutate. Icosabutate was generally well tolerated.

CONCLUSION: Treatment with icosabutate once daily significantly reduced TG, very low–density lipoprotein cholesterol, and Apo C-III levels in patients with very high TG levels. This trial was registered at www.clinicaltrials.gov as NCT01893515.

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Introduction

Hypertriglyceridemia (HTG) is a common lipid abnormality that is often seen in individuals with overweight or obesity, especially when associated with adiposopathic consequences leading to insulin resistance, metabolic syndrome, or type 2 diabetes mellitus.[1,2] Severe HTG (triglyceride [TG] levels ≥500 mg/dL) may have a monogenic etiology caused by rare genetic variants but is more often the result of a complex interplay between genetic susceptibilities and secondary contributors from lifestyle and comorbidities. Severe HTG can increase the risk of pancreatitis, which confers substantial morbidity, potential mortality, and has a high risk of recurrence.[3] Thus, among patients with severe HTG, clinical practice guidelines prioritize TG-lowering therapy to mitigate the risk of pancreatitis.[4,5] In addition to increasing the risk of pancreatitis, HTG also signals elevated levels of atherogenic TG-rich lipoproteins and non–high-density lipoprotein cholesterol (non–HDL-C), which in turn drive the risk of atherosclerotic cardiovascular (CV) events.[6] Once stabilized on statin therapy, further reductions in non–HDL-C are often addressed by TG-lowering strategies. Beyond lifestyle interventions and management of secondary causes, therapeutic options for the management of HTG include prescription omega-3 fatty acid (OM3-FA) agents, fibrates, and niacin.[1]

Three prescription OM3-FAs (eicosapentaenoic [EPA] acid and docosahexaenoic acid [DHA]) are approved for the management of severe HTG. One is a combination of EPA and DHA in ethyl ester form to be taken either as a 4-gram dose (4 capsules) once daily or a 2-gram dose (2 capsules) twice daily. In a contemporary severe HTG patient population, this formulation has been shown to lower TGs by 27% vs baseline and 14% vs control.[7] A meta-analysis of 2 smaller studies conducted 2 decades ago in a somewhat different patient population (ie, higher baseline TG levels and no concomitant statin therapy) reported reductions of 45% and 51% vs baseline and control, respectively.[8] Another OM3-FA product is a combination of EPA and DHA in free fatty acid form administered as 2 to 4 capsules of 1 gram each per day. In a pivotal clinical trial, doses of 4 grams daily reduced TGs by 31% and 21% vs baseline and control, respectively.[9] Finally, there is an EPA-only product, which is administered as 2 gram (2 capsules) twice daily and in a pivotal clinical trial lowered TGs by 27% and 33% vs baseline and control, respectively.[10] Even with these reductions, as much as 50% of patients enrolled in the studies do not achieve a TG level below 500 mg/dL with 12 weeks of therapy.[9,11] All prescription OM3-FA are generally safe and well tolerated, although they may be associated with a mild (and transient) increase in glucose levels, and some patients experience mild gastrointestinal (GI)-related side effects.[10,12]

Icosabutate is a new chemical entity and structurally enhanced fatty acid made by alterations to the EPA molecule with the rationale that addition of an ethyl group in the alpha position and a heteroatom in the beta position will prevent beta-oxidation and complex lipid incorporation and thus ensures increased exposure toward fatty acid–responsive intracellular signaling pathways.[13,14] Based on these changes, it is hypothesized that treatment with icosabutate compared with naturally occurring OM3-FA will result in: (1) improved efficacy in TG lowering; (2) increased potency potentially allowing for once-a-day dosing at a lower dose, and (3) preserved safety. In a mice model of dyslipidemia, when given at a tenth of a human equivalent dose of Omacor (EPA and DHA) at 0.3 mmol/kg vs 3 mmol/kg, icosabutate produced significantly larger TG reductions and also substantially reduced atherogenic cholesterol.[15] In another study in the same model, icosabutate reduced apolipoprotein C-III (Apo C3) expression and increased hepatic low-density lipoprotein (LDL) receptor expression suggesting at least 2 independent lipid-altering modalities. These preclinical observations proved to be consistent with results from an exploratory phase 1b study in hypercholesterolemic subjects where icosabutate significantly reduced TGs, Apo C3, and low-density lipoprotein cholesterol (LDL-C).[16]

The objectives of this study were to evaluate the efficacy and safety of icosabutate 600 mg once daily vs placebo in reducing TG levels, as well as to evaluate its effects on other lipid parameters in subjects with severe HTG (TG levels ≥ 500 mg/dL and ≤1500 mg/dL).

Materials and methods

Study design and organization

This was a randomized, double-blind, placebo-controlled study conducted at 40 sites in the United States. The study comprised a 4- to 6-week diet, lifestyle, and medication stabilization/washout period; a 2-week TG qualifying period; and a 12-week randomized, double-blind treatment period (Fig. 1). At the screening visit, all subjects received counseling regarding the National Cholesterol Education Program Therapeutic Lifestyle Changes diet. The duration of the stabilization period was 4 weeks for subjects who were not on lipid-altering therapy or were on a stable dose of statin (with or without ezetimibe), and 6 weeks for patients who were required to discontinue other lipid-altering therapies not allowed in the study (eg, omega-3, fibrates, and niacin).

The study was conducted according to guidelines of Good Clinical Practice, the Declaration of Helsinki (2000), and the United States 21 Code of Federal Regulations. The appropriate national and institutional regulatory and ethical boards approved the protocol and the informed consent document before initiation of the study. Participants underwent the informed consent process, as evidenced by a signed informed consent document before any study procedure was initiated. The trial was designed by the sponsor (Pronova BioPharma) and Medpace Inc. Medpace

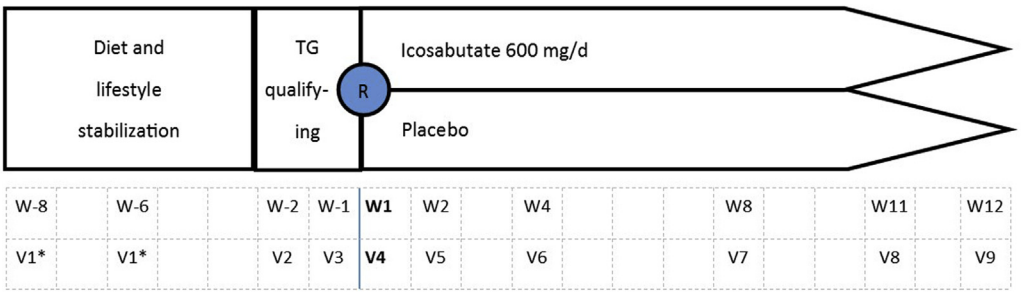

Figure 1 Study design. Eligible subjects had an average fasting triglyceride level of ≥500 and ≤1500 mg/dL to enter the treatment period. Dietary counseling on visits 1 and 4. A lipid profile was obtained at all visits or ET. Adverse events were assessed at visit 2 through visit 9 or ET. *Visit 1 was 6 or 8 weeks before random assignment; 8 weeks for patients who required washout of their current lipid-altering. ET, early termination; TG, triglyceride; V, visit; W, week.

coordinated, monitored, and provided site and data management for the trial. Recruitment began in July 2013, and the study was completed in June 2014. The trial was registered at www.clinicaltrials.gov as NCT01893515.

Study participants

Inclusion study criteria included men and nonpregnant women between 18 and 79 years of age with an average fasting TG level ≥ 500 mg/dL and ≤1500 mg/dL based on the average (arithmetic mean) of the visits 1 and 2 in the TG qualification period. If a subject's average TG level fell outside the required range for entry into the study, then, an additional sample for TG measurement was to be collected 1 week later. If a third sample was collected, then, similar to the average based on the first 2 visits, the entry criteria required the TG level to be within the specified range based on the average of the TG values from the last 2 visits.

Subjects on stable lipid-lowering statin therapy (with or without ezetimibe) and subjects not on non-statin lipid-lowering therapy were eligible to enroll in the study. If determined safe to do so by the investigators, non-statin lipid-altering therapies were discontinued at screening. Subjects were required to agree to maintain a stable diet, smoking habits, and physical activity level throughout the study.

Exclusion criteria included patients with diagnosed lipoprotein lipase impairment or deficiency (Fredrickson type I), apolipoprotein C2 deficiency, or familial dysbetalipoproteinemia (Fredrickson type III); acute or chronic pancreatitis; symptomatic gallstone disease (unless previously treated with cholecystectomy); diagnosed hereditary or acquired myopathy; cancer in the last 5 years (basal or squamous cell skin carcinoma was allowed); type 1 diabetes; uncontrolled type 2 diabetes (glycosylated hemoglobin [HbA1c] > 9.5%); body mass index > 40 kg/m^2; previous bariatric surgery; any ocular disorder for which topical ocular therapy was in use or chronically prescribed; nephrotic range proteinuria, requirement for peritoneal dialysis or hemodialysis; uncontrolled hypertension; clinical evidence of hypothyroidism, or thyroid hormone therapy that was not stable for ≥6 weeks before screening; positive test for human immunodeficiency virus, hepatitis B, or hepatitis C; history or evidence of major and clinical significant disease that could have interfered with the conduct of the study; and patients with any CV event (stroke, myocardial infarction, life-threatening arrhythmia, coronary revascularization) within past 6 months before study enrollment.

Exclusionary laboratory results included thyroid-stimulating hormone >2.0 × upper limit of normal (ULN); alanine aminotransferase or aspartate aminotransferase >2.5 × ULN (unless exercise-related); unexplained creatine kinase concentration >5 × ULN; or creatine kinase elevation due to known muscle disease. Exclusionary medications included weight management pharmacotherapies known to affect intestinal fat absorption within 4 weeks of screening; insulin; chronic prescription pharmacotherapy for metabolic or CV disease management or risk factor modification (eg, antihypertensive and antidiabetes medications) that were not stable for ≥4 weeks before screening; HIV-protease inhibitors, cyclophosphamide or isotretinoin; tamoxifen, estrogens, progestins, or topical testosterone that had not been stable for ≥4 weeks before screening; use of systemic corticosteroids at screening or planned use during the study.

Study drug, randomization, and blinding

Icosabutate is a polyunsaturated fatty acid ether, constructed from EPA alcohol ([5Z,8Z,11Z,14Z,17Z]-icosa-5,8,11,14,17-pentaen-1-ol) and 2-bromo butyric acid (Fig. 2). Subjects were randomly assigned in a 1:1 ratio to 600 mg icosabutate (Pronova BioPharma Norge AS/BASF) or matching placebo (Miglyol 812 [medium-chain fatty acid]) at treatment initiation. Icosabutate and placebo were administered once daily, orally as 6 capsules (100 mg).

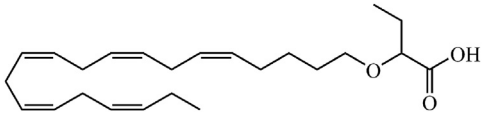

Figure 2 The structure of the icosabutate molecule.

Subjects were instructed to take their medication at approximately the same time each day (eg, morning, mid-day, or evening). On days when the subjects were scheduled for study site visits, the dose of study drug was to be administered by site personnel after the collection of all fasting blood samples. Randomization was stratified by the use of the Medpace ClinTrak Interactive Voice Response System (CTIVRS). Stratification was by baseline TG level ($\leq$700 mg/dL or $>$700 mg/dL), statin use at randomization, and gender. Subjects and investigators were blinded to the treatment assignment, and subjects received blinded study drug. Blinded study drug was packaged and numbered as a kit for each subject based on the randomization scheme. The investigator or designee contacted CTIVRS to randomize the subject and obtain the appropriate double-blind kit identification. The subject randomization number assigned by CTIVRS corresponded to the number on the kit. Study drug was dispensed in amounts exceeding the amount required for the period of time until the next visit. Subjects were instructed to return all unused study drugs at the next visit. Compliance to the study drug regimen was evaluated by counting unused capsules.

Study end points

The primary efficacy parameter was TG percentage change from baseline to end of study (week 12). Secondary end points included non–HDL-C, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), LDL-C, very low–density lipoprotein cholesterol (VLDL-C), remnant-like protein cholesterol (RLP-C), Apo C3, apolipoprotein B (Apo B), apolipoprotein A1 (Apo A1), lipoprotein (a) (Lp [a]), lipoprotein-associated phospholipase A2 (Lp-PLA2), high-sensitivity C-reactive protein (hs-CRP), red blood cell membrane content of EPA and DHA, and metabolic parameters such as hemoglobin A1c (HbA1c), insulin resistance indices, and fasting plasma insulin and glucose.

Laboratory efficacy and safety assessments

All laboratory measurements were performed by Medpace Reference Laboratories (Cincinnati, OH). Efficacy measurements included serum concentration of lipids, including TG, TC, HDL-C, LDL-C, and calculated non–HDL-C (TC–HDL-C), Apo A1, ApoB, and Apo C3, Lp(a), Lp-PLA2, RLP-C, hs-CRP, EPA, DHA content, fasting plasma glucose (FPG), fasting plasma insulin, and HbA1c.

LDL-C was measured via preparative ultracentrifugation (PUC) followed by analysis of the density $>$1.006 fraction on the Beckman Coulter AU Series Chemistry Analyzer. VLDL-C was calculated by subtracting the PUC-derived density $>$ 1.006 fraction produced by PUC from the TC value measured from the Beckman Coulter AU Series Chemistry Analyzer. FPG, TC, and TGs were measured on a Beckman Coulter AU Series Chemistry Analyzer. HDL-C analyses were performed by dextran sulfate precipitation followed by analysis of the supernatant on a Beckman Coulter AU Series Chemistry Analyzer. Apo B, A1, and hs-CRP were measured using rate nephelometry on a Siemens BNII. Apo C3 and RLP-C were measured on the Randox Daytona Analyser. Lp (a) was measured using an immunoturbidimetric assay on a Beckman Coulter AU Series Chemistry Analyzer using an isoform-independent method. Lp-PLA2 analysis was performed by Atherotech Diagnostic Laboratory using the DYNEX-DSX Automated ELISA System. HbA1c was measured on the Tosoh G7/G8 automated high performance liquid chromatography analyzer. Plasma insulin was measured on the Roche Immuno-Analyzers. RBC fatty acid composition was analyzed by gas chromatography (GC) with flame ionization detection. GC was carried out using a GC2010 Gas Chromatograph (Shimadzu Corporation, Columbia, MD) equipped with an SP2560, 100-m fused silica capillary column (0.25 mm internal diameter, 0.2 μm film thickness; Supelco, Bellefonte, PA).

Laboratory safety assessments included serum chemistry, hematology, and urinalysis. A physical examination was performed at screening, randomization, and end of treatment. An off-site ophthalmologic examination was performed after eligibility was confirmed and before randomization and at week 11 or end of treatment. The ophthalmologic investigation was included due to an observation of corneal pitting in a 4-week toxicology study in rat, although this finding could not be replicated in other studies in either rat or other species. Vital signs (heart rate and blood pressure) as well as weight and waist circumference were also measured at every visit. A 12-lead ECG was performed after the screening period, at week 2, and at end of treatment. Adverse events (AE) were assessed at visit 2 after the screening period and through the treatment period.

Statistical analyses

The efficacy analyses included primary and secondary efficacy parameters compared with placebo at week 12. The primary efficacy analysis was based on the intention-to-treat population, which consisted of all randomized subjects who took at least 1 dose of investigational product, had a baseline efficacy measurement, and had at least 1 post-randomization efficacy measurement. The efficacy analysis was repeated on the per-protocol population to test the robustness of the intention-to-treat analysis. Baseline and demographic characteristics were summarized for the randomized population, which included all subjects who signed the informed consent form and were assigned a randomization number. All safety analyses were conducted based on the safety population, which was defined as all randomized subjects who received at least 1 dose of the study drug.

A sample size of 37 completed subjects per treatment group was projected to provide 80% power to detect a difference of 30% between icosabutate and placebo in percent change from baseline in fasting TG levels,

assuming a standard deviation of 45% in TG measurements and a significance level of .05. To accommodate the withdrawal of subjects from randomization to completion of the double-blind treatment period, a total of 86 randomized subjects were planned.

The primary efficacy analysis was performed using an analysis of covariance model with treatment, gender, and the use of statin therapy at randomization as factors and baseline TG value as a covariate. For the primary analysis with parametric methods, the statistical modeling assumptions were examined. If significant departures from normality and/or homogeneity of variance were observed, a new analysis was performed on the overall relative ranks of the efficacy variable. As almost all data significantly departed from normality, nonparametric results are presented. Hodges–Lehman asymptotic 95% confidence intervals were generated and are the primary tests of statistical significance. Wilcoxon rank-sum P values were also generated as a secondary significance measure. No cases of discordance between the Hodges–Lehman confidence interval and the Wilcoxon rank-sum P values were observed.

Statistical programming and analyses were performed with SAS version 9.3.

Results

Of the 352 screened, 87 subjects were randomized to the study: 44 to placebo and 43 to icosabutate (Fig. 3). Six patients in the icosabutate group withdrew from the study, compared with 2 in the placebo group. Of the 6 patients who withdrew from the icosabutate group, 1 was due to an adverse experience ("jittery feeling"), 1 was lost to follow-up, 2 moved to another state, 1 choose to participate in another study, and 1 did not provide a reason.

Demographic and baseline characteristics of randomly assigned subjects are summarized in Tables 1 and 2. The study population included mostly Caucasian men with a mean age of 52 years. Baseline characteristics of the study population were generally comparable. The same proportion of subjects (21%) in both groups maintained statin therapy throughout the study. Mean percent compliance to study medication was 96.5% for placebo and 99.9% for icosabutate.

Table 2 summarizes baseline, end-of-treatment and percentage change or change from baseline for the primary and secondary end points. The median percentage change in TG from baseline was −51.4% for the icosabutate group and −17.1% for the placebo group, with a placebo-corrected change of −32.8%. The TG reduction observed with icosabutate was achieved quickly, and the effect was sustained throughout the treatment period (Fig. 4). In total, 88% of the subjects in the icosabutate treatment group achieved an end-of-study TG value below 500 mg/dL compared with 37% in the placebo group ($P < .001$). The corresponding numbers for those achieving values below 200 mg/dL were 22% for icosabutate and 0% for placebo ($P = .001$).

Compared with placebo, icosabutate significantly reduced VLDL-C (−35.7%, $P < .001$), VLDL-TG (−34.0%, $P < .001$), RLP-C (−33.5%, $P < .001$), and Apo C3 (−34.8%, $P < .001$) and significantly increased HDL-C (18.3%, $P < .001$), LDL-C (28.4% $P < .001$), and trended toward increased Lp (a) (10.2%, $P = .054$). Compared with placebo, icosabutate treatment trended toward reduced non–HDL-C (−7.1%, $P = .064$) and did not differ with regard to changes in TC, Apo B, Apo A1, and Apo B/Apo A1 ratio. Regarding inflammatory markers, compared with placebo, icosabutate significantly reduced Lp-PLA2 (−13.6%, $P = .003$) but did not significantly reduce C-reactive protein (CRP; −0.7 mg/dL, $P = .16$). Compared with placebo, icosabutate significantly increased plasma EPA (58.3%, $P < .001$), but not DHA. The effects on glucose metabolism are summarized in Table 3. Although FPG and HbA1c did not significantly change, compared with placebo, icosabutate reduced fasting plasma

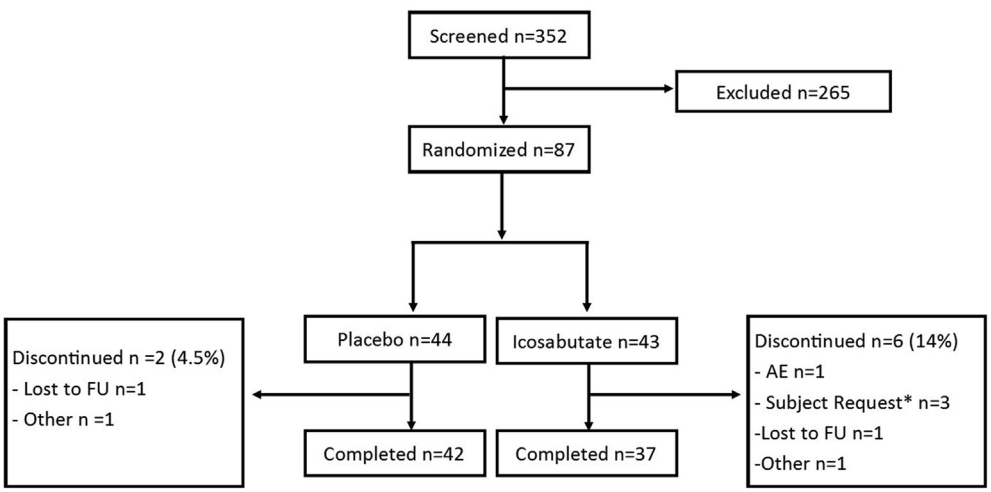

Figure 3 Subject disposition. *Discontinuation due to request; 2 subjects moved to another state, 1 subject discontinued to enroll in another clinical trial. AE, adverse event; FU, follow-up.

Table 1 Demographic and baseline characteristics of all subjects randomly assigned treatment

Characteristics category/statistic	Placebo (n = 44)	Icosabutate, 600 mg (n = 43)
Age (y, mean ± SD)	51.6 ± 11.39	53.5 ± 8.79
Male, n (%)	31 (70.5)	29 (67.4)
Race, n (%)		
Asian	1 (2.3)	0 (0.0)
Black/African American	2 (4.5)	2 (4.7)
White	39 (88.6)	40 (93.0)
Other	2 (4.5)	1 (2.3)
Ethnicity, n (%)		
Hispanic or Latino	8 (18.2)	8 (18.6)
Body mass index, kg/m^2, mean ± SD	32.3 ± 4.56	31.7 ± 4.40
Diabetes, n (%)	17 (38.6)	18 (41.8)
Hypertension, n (%)	25 (56.8)	21 (48.8)
Systolic blood pressure (mm Hg, mean ± SD)*	130.4 ± 12.55	129.5 ± 13.40
Diastolic blood pressure (mm Hg, mean ± SD)*	82.8 ± 8.72	80.6 ± 9.35
On statin at randomization, n (%)	9 (20.5)	9 (20.9)

SD, standard deviation.

Baseline was defined as the randomization visit (week 1) measurement. If the measurement at this visit was missing, the last measurement before the first dose of randomized study drug was used.

*Safety population.

insulin (−5.80 mIU/L, $P = .001$), which translated into a significant reduction in homeostasis model assessment of insulin resistance (−2.1, $P = .006$).

A summary of treatment-emergent AEs (TEAEs) experienced by ≥3% of subjects in either treatment group according to body system and preferred term is summarized in Table 4. During the study, 63% of the subjects had at least 1 TEAE: 67% in the placebo group and 58% in the icosabutate group. In total, 24% subjects had a TEAE considered by investigators to be related to study drug: 21% in the placebo group and 28% in the icosabutate group (Table provided in Appendix). No subjects died during this study. One subject in the placebo group had a serious AE (acute retrocecal appendicitis) during the screening period and recovered from this event before randomization. No serious AEs occurred among the icosabutate-treated subjects. An offsite assessment by an ophthalmologist was performed at screening and at the end of the study, and there were no signals of any ocular side effects related to the treatment. In total, there were 5 eye-related TEAEs in each treatment arm. One subject in the icosabutate group discontinued from the study due to a TEAE ("jittery feeling") considered by the investigator to be related to study drug and, which resolved after discontinuation. Most of the TEAEs during the double-blind treatment period were mild or moderate in severity. No clinically meaningful changes in safety laboratory parameters, ECG parameters, vital signs, or physical examination findings were noted.

Discussion

This was a randomized, double-blind, placebo-controlled proof-of-concept study of icosabutate 600 mg once daily in patients with severe HTG (TG levels ≥ 500 mg/dL and ≤1500 mg/dL). The study results met the primary end point of greater TG lowering from baseline compared with placebo. The median change in TG levels from baseline was −51.4% for the icosabutate group and −17.1% for the placebo group, with a placebo-corrected change of −32.8%. Compared with placebo, icosabutate significantly reduced VLDL-C, VLDL-TG, RLP-C, and Apo C3 and significantly increased HDL-C and LDL-C levels. Non–HDL-C trended downward, Lp (a) trended upward, and no significant changes were found in TC, Apo B, and Apo A1. FPG was unchanged, whereas fasting plasma insulin was significantly reduced with icosabutate. Regarding inflammatory markers, compared with placebo, icosabutate significantly reduced Lp-PLA2, whereas CRP was not significantly reduced. Treatment with icosabutate was generally well tolerated.

The TG reductions on icosabutate therapy were fully realized at the first follow-up visit at 2 weeks, and the effect was sustained throughout the 12-week treatment period. Approximately 9 of 10 patients treated with icosabutate reached a TG level below 500 mg/dL vs 4 of 10 among subjects administered placebo. Although this study did not include any active comparators, the degree of TG reduction with the once-a-day 600-mg icosabutate preparation (15% of a 4-gram dose) was least as effective, or perhaps more efficacious, than the reported effects of 4 grams (4 capsules per day) of approved prescription OM3-FA preparations.[9,10,17] Whether icosabutate provides superior efficacy compared with existing TG-lowering therapies can only be determined in randomized head-to-head trials.

The reductions in Apo C3 with icosabutate were 2 to 3-fold larger than previously observed with prescription

Table 2 Median percent change in lipid and inflammatory parameters from baseline to week 12 by treatment group (intention-to-treat population)

					Icosabutate vs Placebo	
Variable* (mg/dL)	n (1)	Baseline median (Q1, Q3)	Wk 12 median (Q1, Q3)	Change (%) from baseline median (Q1, Q3)	Median (asymptotic 95% Hodges–Lehmann CI)	Wilcoxon rank-sum *P* value
TG						
Placebo	43	687.5 (595.5–892.0)	590.0 (445.0–773.5)	−17.1 (−40.3 to 8.4)	−32.8 (−46.7 to −20.0)	<.0001
Icosabutate	41	610.5 (542.5–878.0)	314.0 (204.0–406.0)	−51.4 (−66.8 to −32.4)		
VLDL-C						
Placebo	43	118.5 (101.5–148.0)	100.0 (73.5–145.0)	−19.7 (−42.7 to 20.1)	−35.7 (−51.5 to −22.4)	<.0001
Icosabutate	41	105.5 (94.5–152.5)	50.5 (35.5–67.5)	−50.9 (−68.2 to −34.9)		
VLDL-TG						
Placebo	40	566.0 (422.0–868.0)	511.5 (379.5–740.0)	−21.7 (−49.7 to 15.9)	−34.0 (−55.2 to 18.5)	<.0001
Icosabutate	40	502.5 (367.0–830.0)	226.0 (146.0–305.5)	−53.3 (−68.8 to −41.6)		
Non–HDL-C						
Placebo	43	206.5 (179.5–246.0)	188.5 (165.5–243.5)	−1.6 (−13.1 to 9.3)	−7.1 (−16.2 to 0.3)	.0640
Icosabutate	41	225.5 (190.0–264.5)	195.0 (167.5–221.0)	−8.1 (−19.0 to 3.6)		
HDL-C						
Placebo	43	31.0 (26.0–33.0)	29.0 (27.0–34.0)	4.0 (−5.0 to 11.5)	18.3 (11.0–25.0)	<.0001
Icosabutate	41	32.0 (29.0–37.0)	38.0 (34.0–45.0)	23.8 (9.7–32.4)		
Total-C						
Placebo	43	234.5 (211.0–292.5)	225.0 (197.0–282.0)	−2.3 (−10.5 to 8.5)	−4.1 (−11.0 to 2.2)	.2070
Icosabutate	41	253.0 (215.0–303.5)	237.5 (210.0–265.0)	−5.2 (−17.7 to 5.0)		
LDL-C						
Placebo	43	79.0 (54.5–97.0)	82.0 (64.5–108.5)	14.0 (−6.9 to 24.5)	28.4 (14.7–46.2)	.0002
Icosabutate	41	94.5 (75.0–133.5)	141.0 (120.0–158.5)	42.6 (11.7–71.2)		
RLP-C						
Placebo	39	49.0 (35.0–84.0)	39.0 (24.0–81.0)	−18.8 (−50.0 to 48.6)	−33.5 (−54.6 to −13.6)	.0031
Icosabutate	40	37.5 (30.5–73.0)	19.0 (15.5–27.5)	−49.9 (−68.9 to −21.1)		
Apo A1						
Placebo	43	135.0 (122.0–154.0)	137.0 (120.0–147.0)	−1.3 (−7.7 to 3.5)	4.7 (−0.5 to 9.9)	.0825
Icosabutate	40	140.0 (127.0–150.5)	137.5 (126.0–151.0)	1.8 (−6.3 to 11.4)		
Apo B						
Placebo	43	109.0 (99.0–119.0)	107.0 (96.0–119.0)	−1.8 (−10.1 to 6.1)	2.4 (−4.4 to 10.0)	.4632
Icosabutate	40	126.5 (105.0–155.5)	128.5 (105.5–142.0)	−1.1 (−11.3 to 13.9)		
Apo C3						
Placebo	40	28.0 (23.0–32.8)	27.2 (18.4–31.8)	−4.7 (−23.6 to 23.1)	−34.8 (−46.8 to −22.4)	<.0001
Icosabutate	40	29.8 (23.8–33.5)	15.5 (13.2–19.8)	−41.3 (−53.9 to −25.9)		
Apo B/A1 ratio						
Placebo	43	0.830 (0.660–0.970)	0.810 (0.680–0.900)	−0.020† (−0.080 to 0.060)	0.020 (−0.070 to 0.080)	.6097
Icosabutate	40	0.895 (0.770–1.110)	0.905 (0.770–1.055)	0.010† (−0.215 to 0.105)		
Lp (a)						
Placebo	40	9.0 (5.0–43.0)	11.0 (5.0–33.5)	0.0† (−6.4 to 13.1)	10.3 (0.0–35.0)	.0544
Icosabutate	40	9.0 (5.0–36.5)	13.5 (5.0–64.5)	1.5† (−1.3 to 70.9)		
hs-CRP						
Placebo	40	2.40 (1.70–5.55)	2.60 (1.50–5.50)	−0.30† (−0.80 to 0.70)	−0.70 (−1.50 to 0.20)	.1624
Icosabutate	40	3.00 (1.55–5.20)	1.90 (1.15–4.15)	−0.70† (−1.95 to 0.80)		
Lp-PLA_2						
Placebo	40	251.6 (207.3–287.6)	243.6 (213.1–275.7)	−4.4 (−13.6 to 13.9)	−13.6 (−22.5 to −4.6)	.0025
Icosabutate	39	239.1 (219.0–280.9)	206.0 (181.3–230.0)	−12.8 (−26.4 to −3.4)		

Apo C3, apolipoprotein C-III; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Lp-PLA_2, lipoprotein-associated phospholipase A_2; non–HDL-C, non–high-density lipoprotein cholesterol; Q1, first quartile; Q3, third quartile; RLP-C, remnant-like protein cholesterol; TG, triglycerides; total-C, total cholesterol; VLDL-C, very low–density lipoprotein cholesterol.

Only subjects with non-missing baseline and end point values were included. Baseline was defined as the average of visit 4 (week 0) and the preceding lipid qualifying visit (either visit 3 [week −1] or if it occurred, visit 3.1) measurements. The week 12 end point was the average of visit 8 (week 11) and visit 9 (week 12) measurements. If the measurements at both visits were missing, the last post-baseline measurement during the double-blind treatment period was carried forward as the end point measurement.

*As almost all data departed from normality, the results are presented by the median values.

†Absolute change.

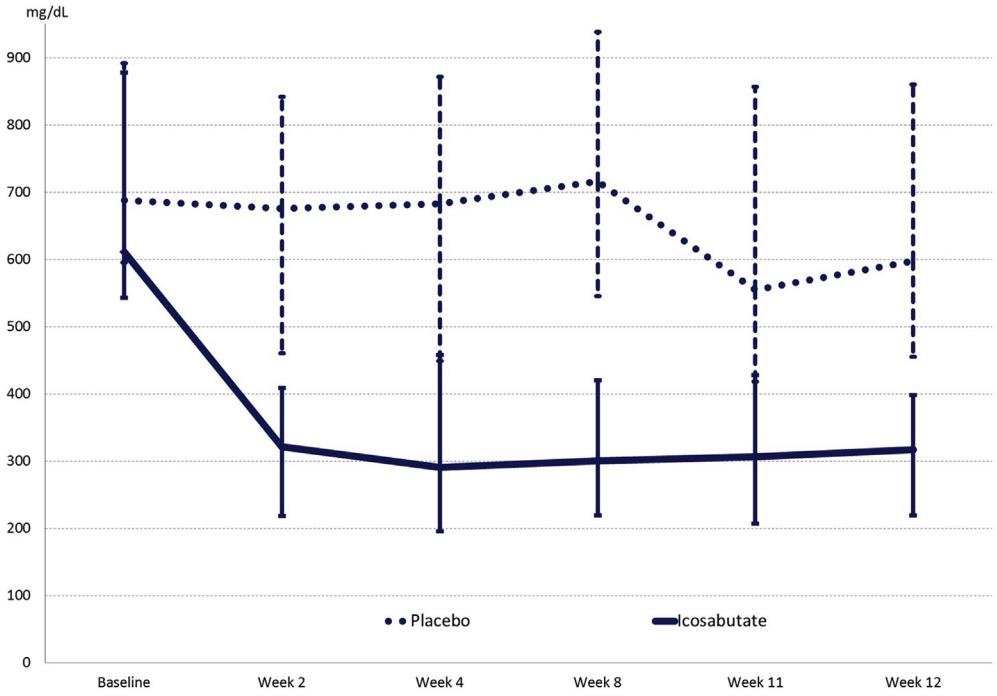

Figure 4 Median triglyceride value (mg/dL) by treatment group at baseline and through week 12.

OM3-FA and may be an important contributor to the large TG-reducing effect.[18,19] Studies with antisense oligonucleotides against Apo C3 synthesis support the concept that Apo C3 is an important proximal determinant of plasma TG levels and related downstream changes in levels of other lipids.[20,21] Apo C3 regulates several key steps in TG metabolism: It promotes hepatic VLDL-TG secretion, inhibits peripheral lipolysis, and reduces hepatic clearance of lipoproteins.[22] Low Apo C3 levels caused by loss-of-function genetic variants have been shown in large-scale Mendelian randomization studies to confer substantially reduced risk of coronary heart disease.[23,24] The Apo C3 reductions seen with icosabutate thus potentially also carry clinical importance from a CV risk reduction standpoint although this hypothesis must be demonstrated in dedicated CV outcome trials.

Table 3 Baseline and end of treatment for measures of glucose metabolism by treatment group (intention-to-treat population)

Variable	n (1)	Baseline (2) median (Q1, Q3)	Wk 12 (3) median (Q1, Q3)	Change from baseline median (Q1, Q3)	Icosabutate vs Placebo Median (asymptotic 95% Hodges–Lehmann CI) *P* value
Fasting plasma glucose (mg/dL)					
Placebo	39	112.0 (93.0–145.0)	119.0 (95.0–161.0)	−1.0 (−12.0 to 12.0)	
Icosabutate	40	116.5 (97.0–145.5)	105.5 (97.5–145.5)	−5.5 (−17.5 to 6.0)	−5.0 (−15.0 to 4.0) .26
HbA1c					
Placebo	40	6.10 (5.65–6.70)	6.15 (5.60–7.05)	0.10 (0.00–0.20)	
Icosabutate	40	5.85 (5.50–7.40)	6.00 (5.55–7.60)	0.10 (−0.10 to 0.30)	0.00 (−0.10 to 0.20) .64
Fasting plasma insulin (mIU/L)					
Placebo	39	21.10 (14.60–34.90)	19.90 (13.60–43.30)	−0.30 (−2.70 to 6.40)	
Icosabutate	40	20.15 (14.65–30.90)	15.70 (12.45–22.95)	−4.80 (−12.00 to −1.40)	−5.80 (−11.40 to −2.30) .001

CI, confidence interval; HbA1c, glycosylated hemoglobin; Q1, first quartile; Q3, third quartile.

Only subjects with non-missing baseline and end point values were included. Baseline was defined as the average of visit 4 (week 0) and the preceding lipid qualifying visit (either visit 3 [week −1] or if it occurred, visit 3.1) measurements. The week 12 end point was the average of visit 8 (week 11) and visit 9 (week 12) measurements. If the measurements at both visits were missing, the last post-baseline measurement during the double-blind treatment period was carried forward as the end point measurement.

Table 4 Treatment-emergent adverse events occurring in >3% of subjects in any treatment group by treatment group (safety population)

System organ class, preferred term	Placebo (N = 43) n (%)	Icosabutate,600 mg (N = 43) n (%)
Eye disorders	5 (11.6)	5 (11.6)
Blepharospasm	1 (2.3)	2 (4.7)
Gastrointestinal disorders	12 (27.9)	9 (20.9)
Diarrhea	4 (9.3)	4 (9.3)
Abdominal discomfort	2 (4.7)	1 (2.3)
Abdominal distension	2 (4.7)	0 (0.0)
Eructation	0 (0.0)	2 (4.7)
Flatulence	2 (4.7)	0 (0.0)
Nausea	0 (0.0)	2 (4.7)
General disorders and administration site conditions	4 (9.3)	3 (7.0)
Fatigue	4 (9.3)	0 (0.0)
Infections and infestations	7 (16.3)	10 (23.3)
Nasopharyngitis	3 (7.0)	2 (4.7)
Urinary tract infection	3 (7.0)	2 (4.7)
Sinusitis	0 (0.0)	3 (7.0)
Upper respiratory tract infection	0 (0.0)	3 (7.0)
Injury, poisoning, and procedural complications	3 (7.0)	0 (0.0)
Ligament sprain	2 (4.7)	0 (0.0)
Musculoskeletal and connective tissue disorders	5 (11.6)	4 (9.3)
Back pain	2 (4.7)	3 (7.0)
Myalgia	2 (4.7)	1 (2.3)
Nervous system disorders	3 (7.0)	3 (7.0)
Dizziness	2 (4.7)	0 (0.0)
Headache	2 (4.7)	0 (0.0)
Skin and subcutaneous tissue disorders	2 (4.7)	4 (9.3)
Pruritus	2 (4.7)	1 (2.3)
Erythema	0 (0.0)	2 (4.7)
Vascular disorders	3 (7.0)	1 (2.3)
Hypertension	3 (7.0)	1 (2.3)

Treatment-emergent adverse events were defined as those adverse events that started on or after the first dose of the study drug or occurred before the first dose of study drug and worsened in severity.

Severe HTG correlates with elevated levels of atherogenic cholesterol and a shift in the distribution of cholesterol away from LDL particles and toward TG-rich particles (ie, VLDL and remnant-like protein) as illustrated by the baseline lipid values of the present study population where the non–HDL-C concentration was more than double the LDL-C levels. Due to the skewed cholesterol distribution in severe HTG, effective TG-lowering interventions often facilitate a redistribution of cholesterol among lipoproteins. Icosabutate reduced VLDL-C and RLP-C by approximately 50% from baseline, whereas LDL-C and HDL-C were increased. These lipid shifts are consistent with a physiological adaptation to an improved TG metabolism as reflected in the reduced levels of TG and Apo C3. Such changes were also observed with Apo C3 antisense in patients with familial chylomicronemia.[20] Importantly, treatment with icosabutate was associated with a trend toward a reduction in non–HDL-C and a partial normalization of the ratio of LDL-C to non–HDL-C. Non–HDL-C reflects cholesterol carried both on LDL particles and TG-rich particles. It therefore captures the totality of the atherogenic burden more accurately than LDL-C; and data from both observational cohorts and randomized studies on statin-treated patients have demonstrated that non–HDL-C is a better predictor of future coronary risk than LDL-C.[25,26] As a result, non–HDL-C is the preferred biomarker of lipid-driven risk according to lipid guidelines.[4]

Icosabutate treatment increased plasma EPA, but not DHA levels, which likely reflects the metabolic degradation of icosabutate. The increase in EPA is much lower than what is observed after treatment with 4 grams of OM3-FA, and so, the increased EPA exposure per se is likely not relevant to the lipid responses observed with icosabutate. In similarity with other OM3-FA preparations, icosabutate likely works through multiple mechanisms. The nuclear transcription factor peroxisome proliferator activator receptor (PPAR) alpha is a key regulator of lipid metabolism and a target of both fibrates and OM3-FA. Icosabutate also has PPAR alpha activity, but with potentially important differences compared with the fibrates and OM3-FA. In vitro, icosabutate has a more than 50-fold higher potency (ie, lower EC50) compared with DHA, whereas receptor transactivation remains sub-maximal at a similar level to DHA and lower than for fibrates. Thus, as a partial PPAR alpha agonist or modulator, icosabutate may not be associated with some metabolic effects observed with the supraphysiological PPAR alpha stimulation achieved with conventional PPAR alpha agonists such as liver enzyme elevations. Icosabutate may also have effects mediated via PPAR alpha–independent mechanisms. Mice dyslipidemia models demonstrate upregulation of sterol regulatory element–binding protein-2 responsive genes and increased hepatic LDL receptor expression. This is supported by observations from an exploratory phase 1 study in hypercholesterolemic patients, where icosabutate lowered LDL-C by approximately 30%. These findings appear to be inconsistent with the LDL-C increase seen in the present study. However, it is likely that when TGs and Apo C3 are lowered from a very high baseline level such as in this trial, the downstream changes in the composition of the non–HDL-C pool will likely counterbalance, and thus mask, any increased hepatic LDL-C clearance.

The incidence of TEAEs was generally similar across the 2 treatment groups. Most events were mild or moderate in severity and not related to study drug as assessed by the investigators. One subject in the icosabutate group

discontinued therapy due to an AE (jittery feeling). Prescription OM3-FA therapies are sometimes associated with an increased, albeit low incidence of GI adverse experiences. In the present study, the incidence of GI AEs was balanced between the treatment groups. No meaningful changes were observed in vital signs, electrocardiographic findings, or clinical chemistry.

Although animal models often support OM3-FA as improving glucose metabolism, this has not been replicated clinically, and some studies of OM3-FA have reported transient increases in glucose levels.[27,28] In this study, FPG and hemoglobin A1c did not change; however, icosabutate significantly reduced fasting plasma insulin. The biological mechanisms behind this finding remain speculative and may involve both PPAR alpha–dependent and –independent pathways. Icosabutate reduces hepatic TG content in rodent dyslipidemia models, which may increase hepatic insulin sensitivity.

Limitations of this study include the demographics, wherein the patient population studied was almost uniformly Caucasian, and the results may not be completely representative of the effects of icosabutate in other demographic groups. However, the racial composition of this trial is similar to that of other trials in patients with very high TG levels.[9,10] Second, this was a proof-of-concept study, with the number of subjects less than expected for a larger phase 3 development program. Third, although of sufficient size and duration to test the primary hypothesis, the study was not powered to detect statistical changes in other end points, such as non–HDL-C and CRP. Finally, this study did not include an active control arm, and without a head-to-head controlled clinical trial, the comparative efficacy of icosabutate vs prescription OM3-FA remains speculative.

In conclusion, in this first investigation to formally test the efficacy of a structurally enhanced fatty acid in subjects with severe HTG, 600-mg icosabutate once daily produced statistically significant and clinically meaningful reductions in TG levels and was generally well tolerated.

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Appendix table Overview of adverse events—safety population

AE category	Placebo (N = 43) n (%)	Icosabutate,600 mg (N = 43) n (%)	Total (N = 86) n (%)
Subjects with any adverse event	32 (74.4)	28 (65.1)	60 (69.8)
Subjects with any TEAE	29 (67.4)	25 (58.1)	54 (62.8)
Maximum severity of TEAE			
Mild	23 (53.5)	11 (25.6)	34 (39.5)
Moderate	5 (11.6)	13 (30.2)	18 (20.9)
Severe	1 (2.3)	1 (2.3)	2 (2.3)
Subjects with any TEAE related to study drug	9 (20.9)	12 (27.9)	21 (24.4)
Maximum severity of TEAE related to study drug			
Mild	9 (20.9)	7 (16.3)	16 (18.6)
Moderate	0 (0.0)	4 (9.3)	4 (4.7)
Severe	0 (0.0)	1 (2.3)	1 (1.2)
Subjects with any SAE	1 (2.3)	0 (0.0)	1 (1.2)
Subjects with any treatment-emergent SAE	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with any SAE related to study drug	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with any AE leading to death	0 (0.0)	0 (0.0)	0 (0.0)
Subjects with TEAE leading to discontinuation or withdrawal	0 (0.0)	1 (2.3)	1 (1.2)
Subjects with TEAE related to study drug and leading to discontinuation/withdrawal	0 (0.0)	1 (2.3)	1 (1.2)

AE, adverse event; SAE, serious adverse event; TEAE, treatment-emergent adverse event.

Note: TEAEs were defined as those adverse events that started on or after the first dose of the study drug or occurred before the first dose of study drug and worsened in severity.

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