

Effect of Recombinant ApoA-I Milano on Coronary Atherosclerosis in Patients With Acute Coronary Syndromes

A Randomized Controlled Trial

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IN A SMALL VILLAGE IN NORTHERN Italy called Limone sul Garda live approximately 40 carriers with a naturally occurring variant of apolipoprotein A-I known as *ApoA-I Milano*. Individuals with ApoA-I Milano are characterized by very low levels of high-density lipoprotein cholesterol (HDL-C) (10-30 mg/dL [0.25-0.78 mmol/L]), apparent longevity,¹ and much less atherosclerosis than expected for their HDL-C levels.² The ApoA-I Milano protein differs from native ApoA-I in that cysteine is substituted at position 173 for arginine allowing disulfide-linked dimer formation. Recombinant ApoA-I Milano has been formulated in a complex

lipid to mimic the properties of nascent HDL (ETC-216, Esperion Therapeutics, Ann Arbor, Mich). Studies in mice and rabbits with experimental ath-

Context Although low levels of high-density lipoprotein cholesterol (HDL-C) increase risk for coronary disease, no data exist regarding potential benefits of administration of HDL-C or an HDL mimetic. ApoA-I Milano is a variant of apolipoprotein A-I identified in individuals in rural Italy who exhibit very low levels of HDL. Infusion of recombinant ApoA-I Milano-phospholipid complexes produces rapid regression of atherosclerosis in animal models.

Objective We assessed the effect of intravenous recombinant ApoA-I Milano/phospholipid complexes (ETC-216) on atheroma burden in patients with acute coronary syndromes (ACS).

Design The study was a double-blind, randomized, placebo-controlled multicenter pilot trial comparing the effect of ETC-216 or placebo on coronary atheroma burden measured by intravascular ultrasound (IVUS).

Setting Ten community and tertiary care hospitals in the United States.

Patients Between November 2001 and March 2003, 123 patients aged 38 to 82 years consented, 57 were randomly assigned, and 47 completed the protocol.

Interventions In a ratio of 1:2:2, patients received 5 weekly infusions of placebo or ETC-216 at 15 mg/kg or 45 mg/kg. Intravascular ultrasound was performed within 2 weeks following ACS and repeated after 5 weekly treatments.

Main Outcome Measures The primary efficacy parameter was the change in percent atheroma volume (follow-up minus baseline) in the combined ETC-216 cohort. Prespecified secondary efficacy measures included the change in total atheroma volume and average maximal atheroma thickness.

Results The mean (SD) percent atheroma volume decreased by -1.06% (3.17%) in the combined ETC-216 group (median, -0.81% ; 95% confidence interval [CI], -1.53% to -0.34% ; $P=.02$ compared with baseline). In the placebo group, mean (SD) percent atheroma volume increased by 0.14% (3.09%; median, 0.03% ; 95% CI, -1.11% to 1.43% ; $P=.97$ compared with baseline). The absolute reduction in atheroma volume in the combined treatment groups was -14.1 mm^3 or a 4.2% decrease from baseline ($P<.001$).

Conclusions A recombinant ApoA-I Milano/phospholipid complex (ETC-216) administered intravenously for 5 doses at weekly intervals produced significant regression of coronary atherosclerosis as measured by IVUS. Although promising, these results require confirmation in larger clinical trials with morbidity and mortality end points.

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erosclerosis have demonstrated that rApoA-I Milano/phospholipid complexes rapidly mobilize cholesterol and thereby reduce atherosclerotic plaque burden. The antiatherosclerotic effects (reductions in plaque lipid and macrophage content) occur in animals as rapidly as 48 hours after a single infusion.³

We hypothesized that short-term weekly infusions of ETC-216 might rapidly regress coronary atherosclerosis in patients following an acute coronary syndrome (ACS). To test this hypothesis, we conducted a prospective, randomized, double blind, placebo-controlled clinical trial of ETC-216 using intravascular ultrasound (IVUS) to measure atheroma burden. Intravascular ultrasound is an imaging modality that provides detailed images of the vessel wall using a high-frequency (40 MHz) miniaturized transducer.⁴ A motorized pullback device is used to generate cross-sectional images throughout the length of the vessel, enabling precise quantification of atherosclerotic disease burden. This approach has been used recently in studies designed to assess the effect of pharmacological agents on atherosclerosis.⁵⁻⁷ We used IVUS to measure change in atheroma volume after a regimen consisting of 5 infusions of ETC-216 or placebo at weekly intervals.

METHODS

Study Protocol

The ApoA-I Milano Trial was a randomized, double-blind, multicenter, parallel-treatment study to assess the effects of 2 different doses of ETC-216 or placebo administered weekly for 5 weeks on coronary atheroma volume as measured by IVUS in patients with ACS. The study was conducted between November 2001 and March 2003. The institutional review boards of all participating centers approved the protocol and written informed consent was obtained from patients prior to any study-related procedures.

Inclusion Criteria

Patients aged 30 to 75 years who required diagnostic coronary angiogra-

phy for clinical indications within 14 days after an ACS, defined as unstable angina, non-ST myocardial infarction, or ST-elevation myocardial infarction, were eligible to be considered for the study. Angiographic inclusion criteria required the presence of an obstructive lesion in a major epicardial vessel with at least a 20% luminal diameter narrowing by visual (angiographic) estimation. The initial IVUS examination was performed in a single coronary artery within 2 weeks of the ACS event. Patients were required to have a target vessel for IVUS interrogation with no more than 50% luminal narrowing throughout a segment with a minimum length of 30 mm (target segment). The target vessel must not have undergone previous angioplasty nor have been a candidate for intervention at the time of baseline catheterization.

The protocol specified that patients receive the customary standard of care for ACS. A core laboratory at the Cleveland Clinic Foundation screened the initial IVUS examination, and the patient was randomly assigned only if the ultrasound study met prespecified image-quality requirements.

Randomization and Allocation Concealment

Patients were randomized to 3 treatment groups in a 1:2:2 ratio—placebo or a low (15 mg/kg) or a high dose (45 mg/kg) of ETC-216. A consulting statistician using SAS version 8.02 (SAS Inc, Cary, NC) generated the randomization sequence prior to the start of the study. A list of randomization assignments for each center was placed in a sealed envelope and sent directly to each center's pharmacist. Patients were enrolled sequentially as they became qualified. Blocks of 5 patients (2:2:1) comprised the code. The pharmacist at each site was unblinded and was aware of allocation prior to randomization but had no other role in the conduct of the study.

Intervention and Blinding

The ETC-216 treatment was prepared as a sterile injectable solution of a recombinant human apolipoprotein A-I

Milano/1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine complex prepared in a 6- μ L phosphate buffer at pH 7.4, 6.4% sucrose and 0.9% mannitol. The ETC-216 treatment was supplied frozen by Esperion Therapeutics Inc, Ann Arbor, Mich, in vials containing protein and phospholipid concentrations in a ratio of 1:1 at approximately 15 mg/mL. The dose calculations were based on the protein content of the dosing solution. The placebo consisted of the 0.9% normal saline. Both active drug and placebo were shrouded in an identical sealed opaque cover. Pharmacists were instructed to deliver the infusate to the study physicians and nurses without revealing the treatment assigned. All other study personnel and the patients had no access to randomization assignments.

The study drug was administered as an intravenous infusion at weekly intervals for 5 doses. Within 2 weeks of the final dose, patients underwent repeat IVUS of the originally imaged coronary vessel to permit comparison of paired examinations at the 2 time points.

Patients were permitted to continue clinically indicated medications for dyslipidemia as long as their doses remained stable for the study duration. Patients in whom lipid-lowering therapy had been initiated or discontinued during the 6 weeks before screening were excluded.

Outcome Measures

The primary outcome measure was change in percent atheroma volume (follow-up minus baseline) as measured by IVUS in both ETC-216 groups combined. Prespecified secondary outcome measures were average change in total atheroma volume, change in average maximal atheroma thickness, and change in atheroma volume in the most severely and least severely diseased 10-mm-long subsegments. Quantitative angiographic change and adverse events were also assessed.

IVUS and Angiography

Following diagnostic coronary angiography, the operator selected a single ma-

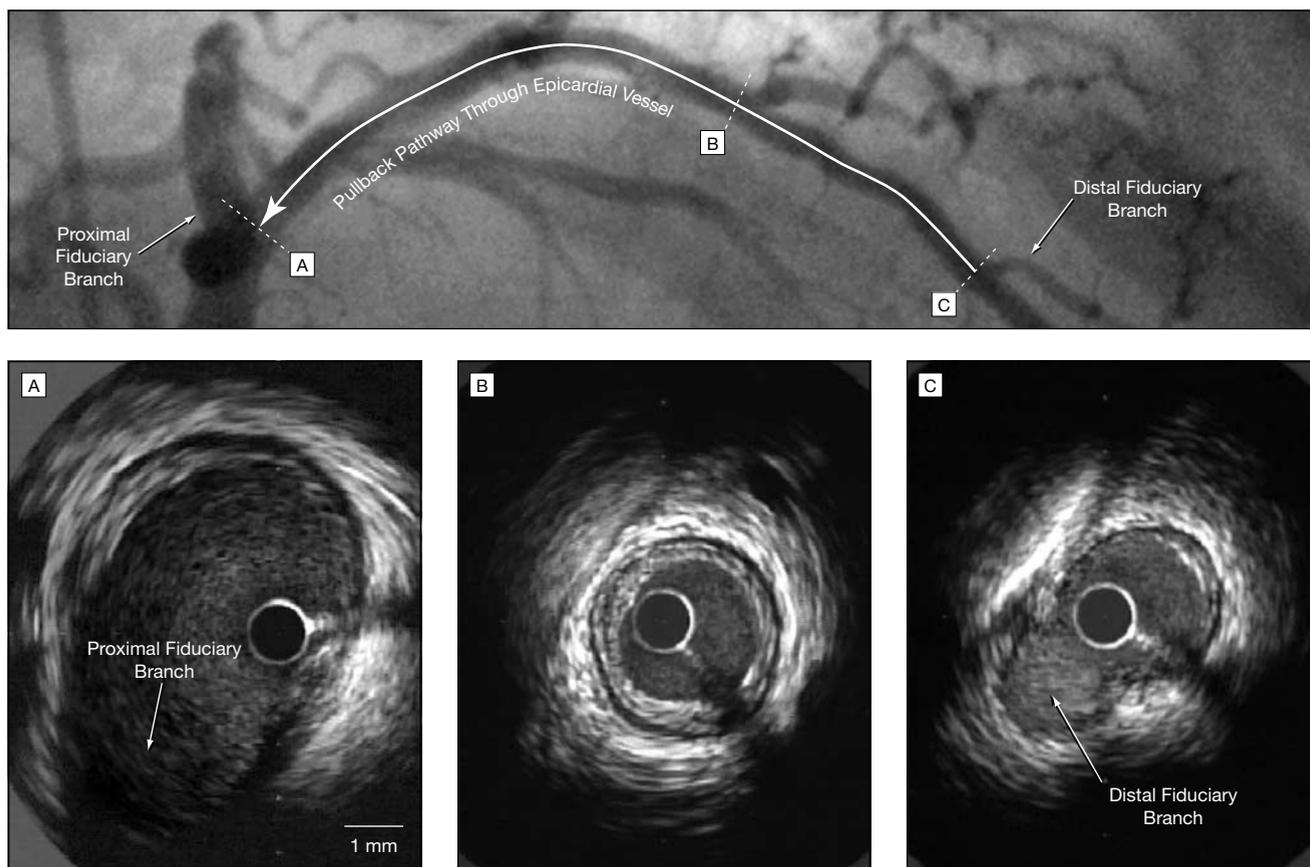
major epicardial vessel for interrogation based on the criteria previously noted. If more than 1 vessel met all inclusion criteria, the investigator was instructed to select the vessel with the longest and least angulated segment suitable for an IVUS pullback. Briefly, after anticoagulation with heparin and administration of 100 μ g to 300 μ g of intracoronary nitroglycerin, a 0.014-inch guidewire was subselectively placed in the vessel selected for interrogation. A 40-MHz, 2.6 F (0.87 mm) IVUS catheter (Atlantis, Boston Scientific Scimed, Inc, Maple Grove, Minn) was advanced into the target vessel and the transducer positioned just distal to a side branch (distal fiduciary site). The IVUS catheter was attached to a motorized pullback apparatus and a dedicated ultrasound scan-

ner (Clearview, Boston Scientific Scimed, Inc). The operator activated a motor drive that progressively withdrew the IVUS transducer at a speed of 0.5 mm per second. During the pullback, IVUS images were obtained at 30 frames per second and recorded on Super-VHS videotape (FIGURE 1). At follow-up, the operator placed the IVUS catheter in the same vessel originally imaged, positioned the transducer just distal to the original fiduciary branch, and initiated a motorized pullback. This procedure ensured that the identical segment was analyzed at baseline and follow-up.

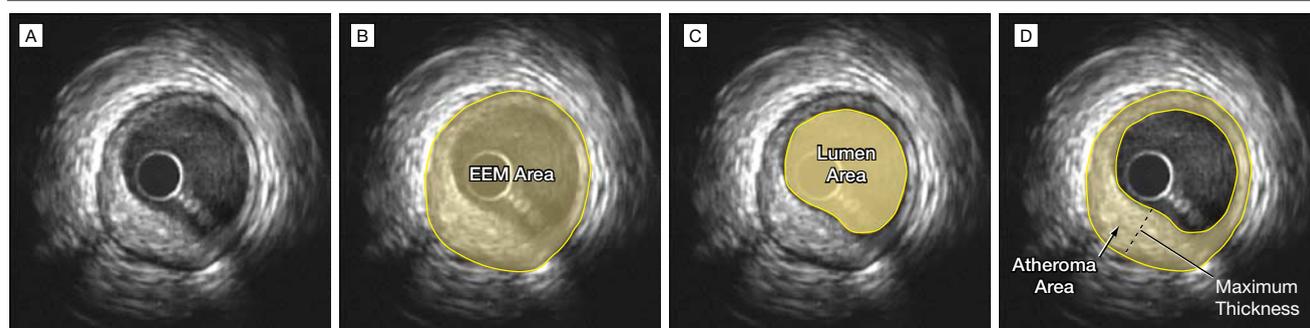
IVUS Core Laboratory Analysis. Videotapes containing the IVUS pullbacks were analyzed in the core laboratory by a single operator (T.T.) blinded to all patient characteristics.

The methods for analysis have been previously described.⁵ Briefly, the operator digitized the videotape, reviewed the pullback, and selected the origin of the most distal side-branch as the beginning point for analysis (Figure 1). Subsequently, every 30th image was selected for analysis, representing a series of cross-sections spaced exactly 0.5 mm apart. The final analyzed cross-section was the most proximal image in the sequence prior to appearance of the left main coronary artery or right coronary ostium (proximal fiduciary site). In this fashion, a series of slices were defined at 0.5-mm intervals over a pullback length of 30 mm to 80 mm (Figure 1). The procedure was repeated for the follow-up examination using identical landmarks to ensure that

Figure 1. Method of Intravascular Ultrasound (IVUS) Interrogation



A motorized pullback of the IVUS imaging catheter is performed beginning at a distal fiduciary branch (site C) and ending at a proximal branch (site A). The pathway of the catheter is illustrated in the angiogram in the top panel. The analysis technician locates the distal branch in the IVUS image, C, and obtains cross-sections every 0.5 mm until a proximal branch is reached, A. An intermediate cross-section is illustrated in B.

Figure 2. Method of Intravascular Ultrasound (IVUS) Measurement

The cross-section for analysis is identified, A; the external elastic membrane (EEM), B, and the lumen area are planimeted, C. Subtraction of the cross-sectional area of the lumen from the area of the EEM allows calculation of the atheroma area, D. The maximal atheroma thickness is directly measured, D.

the identical segment was analyzed at both time points.

Direct IVUS Measurements. Intravascular ultrasound measurements were performed in accordance with the standards of the American College of Cardiology and European Society of Cardiology.⁸ Using National Institutes of Health Image 1.62 (NIH public domain software), the operator performed a calibration procedure by measuring 1-mm grid marks encoded in the IVUS image by the scanner. For each cross-section, the operator performed manual planimetry to trace the leading edges of the luminal and external elastic membrane borders (FIGURE 2). The maximum atheroma thicknesses were also directly measured. The accuracy and reproducibility of these methods have been previously reported, demonstrating that, after calibration, mean IVUS cross sectional area measurements were within 0.5% of actual dimensions for precision-drilled Lucite phantoms ranging in area from 3.24 mm² to 27.99 mm².⁹ The variability of measurements by multiple observers demonstrated an SD of 2.9%.

Derived IVUS Measurements. Atheroma area was calculated as external elastic membrane (EEM) area minus luminal area. Since image cross-sections were obtained at 0.5-mm intervals, the total atheroma volume could be calculated using the Simpson rule as mean atheroma area multiplied by pullback length in millimeters. The percent atheroma volume was computed as:

$$\frac{\sum \text{atheroma areas}}{\sum \text{EEM areas}} \times 100$$

Quantitative Angiography. Analysis of coronary angiography was performed in a core laboratory at the Cleveland Clinic Foundation using standardized methods designed to reduce measurement variability. Comparison of the diameter of the angiographic catheter tip with its known dimension was used to calibrate the system. The angiographic end point was the change in the mean coronary luminal diameter from baseline to follow-up.

Statistical Methods

In the protocol, the assumptions used for power calculations required randomization of 60 patients in a 1:2:2 ratio to placebo and to the low- and high-dose groups, respectively. The estimated reductions in percent atheroma volume were -2% in the low-dose group, -4% in the high-dose group, and -3% for the combined treatment cohort. Assuming an 80% completion rate, the study would provide 75% power to detect a -3% change in percent atheroma volume in combined treatment groups (assumed SD of 7%). Categorical variables are described using frequencies while continuous variables are reported as mean (SD) values. For the efficacy analyses, the Wilcoxon signed rank test was performed, using SAS Version 8.02. The 95% confidence inter-

vals (CIs) were calculated based on the Wilcoxon signed rank test using the method described by Conover.¹⁰

RESULTS

Patient Population

Between November 2001 and March 2003, 123 patients were screened for inclusion in the study and 57 patients were randomly assigned. A total of 47 patients completed the protocol, 11 in the placebo and 21 in the low-dose and 15 in the high-ETC-216 groups. Of the 10 patients not completing the study, 2 were withdrawn for an adverse event, 3 withdrew consent, and 5 had IVUS studies that were not analyzable (the quality of the baseline IVUS study was considered inadequate by the core laboratory after the patient had been administered the drug; FIGURE 3). The demographic, physical examination, and laboratory characteristics of participants are summarized in TABLE 1.

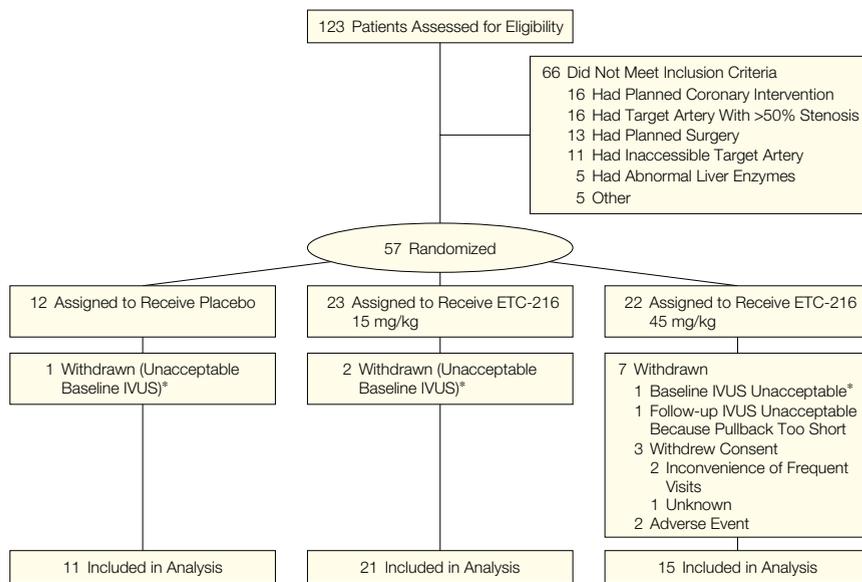
IVUS Baseline Findings

A total of 4016 IVUS cross-sections were analyzed by the core laboratory at both time points and are included in the analyses. The baseline IVUS findings are summarized in Table 1. The mean pullback length was 49.4 mm, containing an average of 86.5 analyzable cross-sections per patient.

Analyses

Primary Efficacy. The primary prespecified end point was the change in per-

Figure 3. Disposition of Patients in the Trial



ETC-216 indicates apolipoprotein A-I Milano/phospholipid; IVUS, intravascular ultrasound.

*These 4 patients received a single dose of study drug or placebo but were withdrawn after their baseline IVUS examination was deemed unacceptable by the core laboratory.

Table 1. Baseline Characteristics*

Characteristic	Placebo (n = 12)	ETC-216	
		15 mg/kg (n = 23)	45 mg/kg (n = 22)
Age, mean (SD) y	60.7 (12.9)	56.8 (7.5)	55.9 (11.8)
Men	6/12 (50)	16/23 (70)	13/22 (59)
White	12/12 (100)	22/23 (96)	21/22 (95)
Weight, mean (SD), kg	80.8 (12.4)	91.0 (16.3)	82.9 (11.7)
Current smoker	4/12 (33)	22/23 (52)	8/22 (36)
Hypertension	10/12 (83)	15/22 (65)	16/22 (73)
Statin use	4/12 (33)	9/23 (39)	12/22 (55)
Diabetes mellitus	3/12 (25)	3/23 (13)	2/22 (9)
Unstable angina	9/12 (75)	15/23 (65)	17/22 (77)
Non-ST-elevation MI	1/12 (8)	7/23 (30)	3/22 (14)
ST-elevation MI	2/12 (17)	1/23 (4)	2/22 (9)
Cholesterol, mean (SD), mg/dL/No. of participants†			
Total	177 (32)/12	185 (34)/22	174 (54)/21
Low-density lipoprotein	118 (25)/4	122 (50)/11	112 (32)/4
High-density lipoprotein	46 (7)/4	38 (10)/11	45 (7)/4
Triglycerides, mean (SD), mg/dL/No. of participants†	212 (153)/10	143 (83)/19	150 (77)/15
Intravascular ultrasound, mean (SD)/No. of participants			
Percent atheroma volume, %*	34.8 (8.4)/11	39.7 (7.0)/21	37.9 (7.8)/15
Total atheroma volume, mm ³ *	173 (113)/11	296 (167)/21	231 (157)/15
Average maximum plaque thickness, mm*	0.65 (0.32)/11	0.82 (0.19)/21	0.74 (0.28)/15
Angiographic coronary luminal diameter, mean (SD), mm	2.28 (0.63)/11	2.41 (0.42)/21	2.37 (0.34)/16

Abbreviations: ETC-216, apolipoprotein A-I Milano/phospholipid; MI, myocardial infarction.

*Data are presented as number/total number of patients (percentage) unless otherwise indicated.

†To convert total, low-density, and high-density cholesterol from mg/dL to mmol/L, multiply by 0.025; to convert triglycerides from mg/dL to mmol/L, multiply by 0.0113.

cent atheroma volume (end of study minus baseline) in the combined ETC-216 treatment group. This group included all patients who were randomized in whom an evaluable final IVUS was obtained. A positive result was defined as a negative change in percent atheroma volume with CIs not including zero. In the combined treatment group (patients who received either the low- or high-dose ETC-216), the change in mean (SD) percent atheroma volume was -1.06% (3.17%). The median was -0.81% (95% CI, -1.53% to -0.34% ; $P = .02$ compared with baseline; TABLE 2). For the placebo group, the mean (SD) change was 0.14% (3.09%). The median was 0.03% (95% CI, -1.11% to 1.43% ; $P = .97$ compared with baseline).

Predefined Secondary Efficacy. Compared with baseline, the mean (SD) change in total atheroma volume in the combined treatment group was -14.1 mm^3 (39.5 mm^3 ; median -13.3 mm^3 ; 95% CI, -20.7 to -7.2 ; $P < .001$). For the placebo group, the corresponding change was -2.9 mm^3 (23.3 mm^3). The median was -0.2 mm^3 (95% CI, -8.6 to 8.2 ; $P = .97$; TABLE 3). The mean (SD) change from baseline in maximum atheroma thickness for the combined treatment group was -0.042 mm (0.080 mm). The median was -0.035 mm (95% CI, -0.058 to -0.020 ; $P < .001$). For the placebo group, the corresponding change was -0.008 mm (0.061 mm). The median was -0.009 (95% CI, -0.035 to 0.026 ; $P = .83$; TABLE 4).

Subsegmental. To determine the interaction between observed drug effects and disease severity, the protocol prespecified analysis of the most severely and least severely diseased 10-mm-long subsegments. For the combined treatment cohort, the effect of ETC-216 was predominantly observed as regression of disease in the most severely diseased 10-mm subsegment ($P < .001$; TABLE 5) In the least severely diseased subsegment, no treatment effect was observed ($P = .49$, data available on request from the author).

Angiographic Results. The prespecified angiographic secondary efficacy end point was the change in mean coro-

nary luminal diameter. Neither the placebo ($P=.63$) nor the combined treatment subgroup ($P=.62$) showed a statistically significant change in coronary luminal diameter comparing follow-up and baseline (data available on request from the author).

Exploratory Analyses. Because of the small size of the trial and numerically greater plaque burden in the actively treated groups, a post hoc sensitivity analysis was performed using several additional methods for evaluating efficacy. These included unpaired comparisons of the combined treatment arm to placebo for the primary and secondary efficacy parameters adjusting for baseline values (Tables 2-5). An addi-

tional analysis was performed in which the patients who were randomly assigned but did not complete the trial were included in the efficacy analyses and assumed to have no change in plaque burden. Using this alternative analysis (imputing patients who did not complete the study), the resulting P value for the primary and secondary efficacy parameters were unchanged.

Adverse Events

Adverse events are noted in TABLE 6. Minor gastrointestinal adverse effects, such as nausea, occurred in all 3 groups. One patient in the high-dose group developed an elevated aspartate aminotransferase on a single occasion ($>3 \times$ the up-

per limit of normal), accompanied by nausea, vomiting, and cholelithiasis and was withdrawn from the study for an adverse event. Another patient in the high-dose group experienced a reaction consisting of chills, nausea, diaphoresis, rigors, vomiting, and mild rash during infusion, deemed possibly drug related and was withdrawn from the study for an adverse event.

COMMENT

Although epidemiological studies have demonstrated that HDL-C levels are inversely correlated with atherosclerotic clinical events, the value of raising HDL-C as a therapeutic target remains uncertain.¹¹ Currently avail-

Table 2. Percent Atheroma Volume in the Target Coronary Segment

Treatment Groups	No. of Patients	Baseline		Follow-up		Change From Baseline		P Value*
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (95% CI)	
Placebo	11	34.80 (8.44)	35.29 (26.99 to 39.81)	34.94 (9.74)	33.59 (27.07 to 41.09)	0.14 (3.09)	0.03 (-1.11 to 1.43)	.97
ETC-216								
15 mg/kg	21	39.71 (7.04)	40.55 (34.16 to 44.97)	38.42 (6.5)	38.84 (32.78 to 42.87)	-1.29 (3.48)	-1.14 (-2.24 to -0.56)	.03
45 mg/kg	15	37.92 (7.83)	41.22 (31.92 to 44.10)	37.19 (7.71)	36.62 (32.81 to 44.02)	-0.73 (2.75)	-0.34 (-1.21 to 0.43)	.45
Combined	36	38.96 (7.32)	40.88 (34.01 to 44.53)	37.91 (6.95)	38.17 (32.79 to 43.53)	-1.06 (3.17)	-0.81 (-1.53 to -0.34)	.02†

Abbreviations: CI, confidence interval; IQR, interquartile range.

* P values for within-group comparison from Wilcoxon signed rank test. For between-group comparison of ETC 216, apolipoprotein A-1 Milano/phospholipid, combined vs placebo from analysis of covariance of ranks of change from baseline, with the baseline value as a covariate, $P=.29$.

†Prespecified primary efficacy end point of the trial.

Table 3. Total Atheroma Volume in the Target Coronary Segment

Treatment Groups	No. of Patients	Baseline, mm ³		Follow-up, mm ³		Change From Baseline, mm ³		P Value*
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (95% CI)	
Placebo	11	172.6 (113.2)	149.0 (58.6 to 216.5)	169.8 (118.0)	145.1 (58.4 to 217.8)	-2.9 (23.3)	-0.2 (-8.6 to 8.2)	.97
ETC-216								
15 mg/kg	21	295.5 (166.5)	279.7 (173.6 to 370.7)	280.4 (155.6)	265.5 (161.1 to 351.6)	-15.1 (50.6)	-15.0 (-29.6 to -4.9)	.02
45 mg/kg	15	230.6 (156.7)	195.8 (90.9 to 332.9)	218.0 (145.6)	183.8 (89.4 to 311.1)	-12.6 (15.3)	-12.0 (-20.6 to -2.9)	.007
Combined	36	268.4 (163.5)	263.7 (129.4 to 359.3)	254.4 (152.6)	244.8 (131.0 to 337.6)	-14.1 (39.5)	-13.3 (-20.7 to -7.2)	<.001†

Abbreviations: CI, confidence interval; IQR, interquartile range.

* P values for within-group comparison from Wilcoxon signed rank test. For between-group comparison of ETC 216, apolipoprotein A-1 Milano/phospholipid, combined vs placebo from analysis of covariance of ranks of change from baseline, with the baseline value as a covariate, $P=.18$.

†Prespecified secondary efficacy end point of the trial.

Table 4. Mean Maximum Atheroma Thickness in the Target Coronary Segment

Treatment Groups	No. of Patients	Baseline, mm		Follow-up, mm		Change From Baseline, mm		P Value*
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (95% CI)	
Placebo	11	0.649 (0.317)	0.683 (0.402 to 0.846)	0.641 (0.332)	0.584 (0.392 to 0.827)	-0.008 (0.061)	-0.009 (-0.035 to 0.026)	.83
ETC-216								
15 mg/kg	21	0.815 (0.194)	0.830 (0.683 to 0.953)	0.771 (0.168)	0.824 (0.646 to 0.865)	-0.044 (0.094)	-0.049 (-0.079 to -0.015)	.03
45 mg/kg	15	0.738 (0.280)	0.792 (0.531 to 0.992)	0.699 (0.269)	0.758 (0.457 to 0.963)	-0.039 (0.059)	-0.029 (-0.056 to -0.007)	.02
Combined	36	0.783 (0.233)	0.816 (0.578 to 0.963)	0.741 (0.216)	0.814 (0.552 to 0.895)	-0.042 (0.080)	-0.035 (-0.058 to -0.020)	<.001†

Abbreviations: CI, confidence interval; IQR, interquartile range.

* P values for within-group comparison from Wilcoxon signed rank test. For between-group comparison of ETC 216, apolipoprotein A-1 Milano/phospholipid, combined vs placebo from analysis of covariance of ranks of change from baseline with the baseline, value as a covariate, $P=.23$.

†Prespecified secondary efficacy end point of the trial.

Table 5. Atheroma Volume in Most Severely Diseased 10-mm Subsegment

Treatment Groups	No. of Patients	Baseline, mm ³		Follow-up, mm ³		Change From Baseline, mm ³		P Value*
		Mean (SD)	Median (IQR)	Mean (SD)	Median (IQR)	Mean (SD)	Median (95% CI)	
Placebo	11	60.7 (28.1)	64.5 (31.4 to 80.8)	60.0 (32.6)	58.4 (29.4 to 79.7)	-0.7 (15)	-0.2 (-5.7 to 8.1)	.97
ETC-216								
15 mg/kg	20	79.0 (24)	80.5 (54.9 to 97.0)	70.4 (25.3)	73.1 (47.3 to 81.8)	-8.6 (15.5)	-4.7 (-16.8 to -3.6)	.02
45 mg/kg	15	67.0 (32.0)	68.3 (34.7 to 102.7)	61.7 (28.3)	65.4 (34.6 to 90.4)	-5.3 (7.1)	-2.9 (-8.8 to -0.8)	.02
Combined	35	73.8 (27.9)	74.7 (50.1 to 97.4)	66.6 (26.6)	70.8 (44.6 to 87.0)	-7.2 (12.6)	-4.1 (-10.8 to -3.2)	<.001†

Abbreviations: CI, confidence interval; IQR, interquartile range.

*P values are for within-group comparison from Wilcoxon signed rank test. For between-group comparison of ETC 216, apolipoprotein A-1 Milano/phospholipid, combined vs placebo from analysis of covariance of ranks of change from baseline, with the baseline value as covariate, P = .13.

†Prespecified secondary efficacy end point of the trial.

Table 6. Adverse Events in the Intent-to-Treat Population

Characteristic	No. (%)		
	Placebo (n = 12)	ETC-216, mg/kg	
		15 (n = 23)	45 (n = 22)
Nausea, vomiting, or abdominal pain	7 (58)	5 (22)	15 (68)
Abnormal liver function test result	0	0	1 (4.5)
Cholelithiasis	0	0	1 (4.5)
Stroke	0	1 (4.3)	0
Headache	2 (16.7)	5 (21.7)	7 (31.8)
Arthralgia	2 (16.7)	1 (4.3)	0
Fluid retention or edema	1 (8.3)	1 (4.3)	1 (4.5)
Possible hypersensitivity reaction	0	0	1 (4.5)

able drugs have only a modest effect on HDL-C and possess other pharmacological effects that confound efforts to determine whether observed benefits are related to alterations in HDL-C levels. Administration of an exogenously produced HDL mimetic offers the opportunity to explore an entirely new approach to atherosclerosis treatment. We used a unique form of synthetic HDL, an ApoA-I Milano/phospholipid complex, because the naturally occurring carriers of ApoA-I Milano are protected from vascular disease. In this initial study, this new strategy had a favorable effect on atherosclerotic disease burden despite a short duration of treatment. An example of regression in a patient treated with ETC-216 is illustrated in FIGURE 4.

The statistical approach used to assess regression in this study merits additional comment. In this type of analysis, each patient serves as his or her own control, with paired comparisons performed between the baseline and end-of-study measures of atheroma burden. Because this was a pilot study, the

study did not have sufficient power to test the hypothesis that significant differences between groups would be identified. However, the protocol did specify analysis of the placebo-treated patients as a separate group to provide insight into any systematic observer bias in measurement of atheroma burden. In addition, an intent-to-treat analysis of all patients analyzed as randomized was not preplanned. However, the results of the study did not change significantly if no change in atheroma burden was assumed for all patients who withdrew before study completion. Based on the findings of all analyses, we believe that the current study provides compelling evidence of atherosclerosis regression following short-term treatment with the exogenous HDL mimetic ETC-216, but these results should be confirmed in a larger, long-term study with clinical end points.

The rapidity and magnitude of the changes in atherosclerotic disease burden observed in the current study have not been previously observed. Brown

et al¹² administered the combination of simvastatin and niacin and reported a -0.4% change in angiographic percent stenosis after 3 years of treatment. The regression observed in our study was substantially larger (-1% for percent atheroma volume and -4.2% for total atheroma volume) and occurred after only 5 weeks of treatment. Several phenomena may account for these observations. Therapy with an HDL mimetic may achieve benefit more quickly and produce a greater extent of regression than conventional lipid-lowering treatment. Alternatively, IVUS measures changes occurring within the vessel wall, not just the lumen. A substantial reduction in atherosclerotic burden may occur in the absence of changes in luminal measurements (Figure 4).

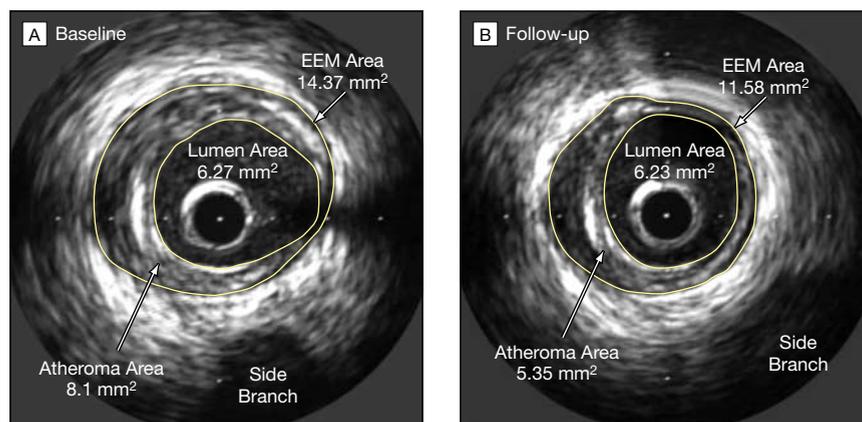
The mechanisms of action of ApoA-1 Milano/phospholipid that result in regression of atherosclerosis are unknown but presumably are related to an increase in reverse cholesterol transport from atheromatous lesions to the serum with subsequent modification and removal by the liver. The cysteine substitution for arginine at position 173 in the ApoA-1 Milano variant allows dimerization, forming large HDL particles that may be particularly active in reverse cholesterol transport. In vitro experiments have demonstrated increased cholesterol efflux from cholesterol-loaded hepatoma cells incubated with serum from ApoA-1 Milano carriers or from transgenic mice.¹³ Because no previous human studies exist from which to determine the optimal dose of an exogenous HDL mimetic, we tested 2 different doses of ETC-216, 15 mg/kg and 45 mg/kg. For the primary

and secondary efficacy analyses, there was no evidence of a greater extent of regression for the higher dose. These data suggest that ETC-216 is capable of enhancing reverse cholesterol transport at both dosage levels.

These data provide intriguing insights into the pathophysiology of coronary atherosclerosis. Previously, this disease was regarded as relatively static, characterized by steady, gradual progression. Trials using angiography and carotid ultrasound to measure atherosclerosis progression typically treated patients for 2 to 3 years.^{12,14-16} The rapid regression observed in our study provides evidence of a more dynamic process. Treatment strategies designed to enhance reverse cholesterol transport may work quickly if sufficiently efficacious. Accordingly, we believe that therapies designed to affect HDL-C represent an important emerging therapeutic target. These findings suggest the potential for a novel strategy for management of the patients with ACS. If these results are confirmed, administration of an agent to stimulate reverse-cholesterol transport could be used in the first few weeks or months following an acute event. After a period of intense treatment, ongoing therapy with conventional lipid modulating agents can provide long-term clinical benefits.¹⁷

The application of IVUS in studies of atherosclerosis regression or progression represents an important emerging trend in research. Intravascular ultrasound is particularly well suited for this application. Current devices operate at very high frequencies, 30 MHz or higher, and offer an axial resolution of less than 150 μm and a temporal resolution of 33 milliseconds.^{4,7} Unlike angiography, IVUS depicts a 360° image of the vessel wall rather than a 2-dimensional projection of the lumen.¹⁸ Precise motorized pullback enables reconstruction of the atheroma burden within long segments of the coronary artery. These properties permit shorter duration studies in smaller numbers of patients than previously possible from conventional angiographic methods. Other recent IVUS studies have demonstrated differences in anti-

Figure 4. Example of Atheroma Regression in a Patient Who Received High-Dose ETC-216



The atheroma area decreased from 8.1 to 5.35 mm² with virtually no change in the lumen area. EEM indicates external elastic membrane.

atherosclerotic treatment strategies with relatively small sample sizes.^{19,20}

This study has several limitations, particularly the small sample size, which limits interpretation of both safety and efficacy data. However, the observation of a large treatment effect and statistically consistent results for the primary and secondary end points is encouraging. Nonetheless, the current result is a proof-of-concept study that must be explored in larger trials. Due to the absence of published data linking IVUS changes in plaque volume to morbidity and mortality, the clinical relevance of changes in disease burden remain uncertain. However, the relationship between coronary disease progression rates and clinical events has been previously established in trials using angiographic end points.²¹ Accordingly, we think it is likely that a rapid and large reduction in disease burden will improve long-term clinical outcome. Nevertheless, demonstration of an effect on major clinical events is necessary to confirm the clinical utility of this approach to the treatment of coronary atherosclerosis.

Despite these limitations, several conclusions from this study are warranted. This initial trial of an exogenously produced HDL mimetic demonstrated significant evidence of rapid regression of atherosclerosis. The potential utility of the new approach must be fully ex-

plored in a larger patient population with longer follow-up, assessing a variety of clinical end points, including morbidity and mortality.

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