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Inhibition of microsomal triglyceride transfer protein alone or with ezetimibe in patients with moderate hypercholesterolemia

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SUMMARY

Background Many patients with coronary heart disease do not achieve recommended LDL-cholesterol levels, due to either intolerance or inadequate response to available lipid-lowering therapy. Microsomal triglyceride transfer protein (MTP) inhibitors might provide an alternative way to lower LDL-cholesterol levels. We tested the safety and LDL-cholesterol-lowering efficacy of an MTP inhibitor, AEGR-733 (Aegerion Pharmaceuticals Inc., Bridgewater, NJ), alone and in combination with ezetimibe.

Methods We performed a multicenter, double-blind, 12-week trial, which included 84 patients with hypercholesterolemia. Patients were randomly assigned ezetimibe 10 mg daily ($n=29$); AEGR-733 5.0 mg daily for the first 4 weeks, 7.5 mg daily for the second 4 weeks and 10 mg daily for the last 4 weeks ($n=28$); or ezetimibe 10 mg daily and AEGR-733 administered with the dose titration described above ($n=28$).

Results Ezetimibe monotherapy led to a 20–22% decrease in LDL-cholesterol concentrations. AEGR-733 monotherapy led to a dose-dependent decrease in LDL-cholesterol concentration: 19% at 5.0 mg, 26% at 7.5 mg and 30% at 10 mg. Combined therapy produced similar but larger dose-dependent decreases (35%, 38% and 46%, respectively). The number of patients who discontinued study drugs owing to adverse events was five with ezetimibe alone, nine with AEGR-733 alone, and four with combined ezetimibe and AEGR-733. Discontinuations from AEGR-733 were due primarily to mild transaminase elevations.

Conclusions Inhibition of LDL production with low-dose AEGR-733, either alone or in combination with ezetimibe, could be an effective therapeutic option for patients unable to reach target LDL-cholesterol levels.

KEYWORDS ezetimibe, hypercholesterolemia, microsomal triglyceride transfer protein inhibitor

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INTRODUCTION

Guidelines on the optimum intensity of LDL-cholesterol lowering have evolved in step with findings from clinical trials. The National Cholesterol Education Program (NCEP) guidelines from 2001 set the target level at below 2.6 mmol/l (100 mg/dl) for high-risk patients with coronary heart disease or its risk equivalent.[1] These guidelines were updated in 2004 to provide an optional therapeutic target of below 1.8 mmol/l (70 mg/dl) for very high-risk patients (those with additional risk factors, such as diabetes).[2] In 2006, the spectrum of patients to which the lower value applied was broadened to include all patients with atherosclerotic disease. Furthermore, a minimum of 30–40% reduction in LDL was recommended for patients at moderate and high risk.[3] Unfortunately, at least 20% of high-risk patients do not achieve these LDL-cholesterol targets, with those in the highest risk group being the least likely to do so.[4] This difficulty might be due partly to statin intolerance. The rate of statin discontinuation owing to adverse events, observed in clinical trials and clinical practice, ranges from 1% to 7% and is mainly caused by myalgias.[5] In a 52-week lipid efficacy study of five different statins, the rate of discontinuations due to adverse events was even higher (4–13%).[6] Furthermore, there are patients for whom high-dose statins are contraindicated, such as those taking amiodarone,[7] or with clinical factors that raise the risk of rhabdomyolysis.[8] Because statin-intolerant patients have few other options for achieving treatment goals, there is an unmet clinical need for additional therapies that can lower LDL-cholesterol levels.

One potential therapeutic target is the assembly and secretion of apolipoprotein B (apoB)-containing lipoproteins. Microsomal triglyceride transfer protein (MTP) is an intracellular lipid-transfer protein found in the endoplasmic reticulum and which is responsible for transferring lipid molecules onto apoB. This transfer forms part of the assembly of triglyceride-rich lipoproteins, such as chylomicrons in the

intestine and VLDL in the liver.[9] Patients with the genetic disorder abetalipoproteinemia have loss-of-function mutations in the microsomal triglyceride transfer protein gene (*MTTP*),[10] resulting in extremely low plasma concentrations of cholesterol and triglycerides and absense of chylomicrons, VLDL and LDL.[11] The elucidation of the mechanistic basis for this disease led to the concept that small-molecule inhibitors of MTP could reduce LDL-cholesterol levels. Indeed, preclinical studies in animal models showed that inhibition of MTP significantly reduced serum cholesterol levels and slowed the formation of atherosclerotic plaques.[12,13] Furthermore, MTP inhibition significantly reduced LDL-cholesterol levels in patients with homozygous familial hypercholesterolemia.[14]

Clinical applications of MTP inhibitors have been focused primarily on high-dose monotherapy to produce substantial reductions in LDL-cholesterol levels (particularly for patients with homozygous familial hypercholesterolemia); however, this strategy has been associated with an unacceptable rate and severity of gastrointestinal and hepatic adverse events, thereby prohibiting its use in a broader population of patients with hyperlipidemia. Because these side effects are thought to be directly linked to the mechanism of MTP-inhibition, we hypothesized that much lower doses would yield clinically useful LDL-cholesterol-lowering results but would be better tolerated. In addition, we also hypothesized that the LDL-cholesterol-lowering effects of the MTP inhibitor AEGR-733 (Aegerion Pharmaceuticals Inc., Bridgewater, NJ; previously BMS-201038, Bristol-Myers Squibb, New York, NY) at these reduced doses would be additive to those of the cholesterol absorption inhibitor ezetimibe, because the two drugs have different mechanisms. To test our hypotheses we evaluated the LDL-cholesterol-lowering efficacy of AEGR-733, both alone and in combination with ezetimibe, in patients with moderate hypercholesterolemia.

METHODS

This clinical trial is registered on Clinicaltrials.gov (registry number NCT00405067 assigned on 28 November 2006).

Study patients

This trial was approved by a central investigational review board (ASPIRE Institutional Review Board LLC, San Diego, CA). Before the study started, all potential participants signed an informed consent form approved by the review board. Hypercholesterolemic patients of 18–70 years of age from six geographically distinct lipid treatment centers within the US were eligible. Patients with 0–1 risk factors were required to have an LDL-cholesterol concentration between 4.1 and 6.5 mmol/l (160 and 250 mg/dl), and those with more than two risk factors were required to have an LDL-cholesterol concentration between 3.4 and 6.5 mmol/l (130 and 250 mg/dl). Baseline LDL-cholesterol was the mean of measurements obtained at the first two clinic screening visits. The main exclusion criteria were uncontrolled hypertension, creatinine levels greater than 221 μmol/l (2.5 mg/dl), liver disease or transaminase levels greater than 1.5 times the upper limit of normal, symptomatic congestive heart failure, diabetes, plasma triglyceride levels greater than 4.5 mmol/l (400 mg/dl), or an acute cardiovascular event within the prior 6 months. Patients receiving concomitant lipid-lowering therapy were required to discontinue these medications 4 weeks before screening and throughout the trial. AEGR-733 was manufactured in accordance with current Good Manufacturing Practices.

Study design

This was a phase II, prospective, randomized, double-blind study. Participants initially underwent a 2–6-week eligibility screening process to assess their ability to follow a low-fat diet (<20% of energy from total fat and <7% of energy from saturated fat) to ensure that lipid values were within the range stipulated in the inclusion criteria and to wash-out any prior lipid-lowering drugs. The active treatment part of the protocol was a 12-week treatment period with interim visits at weeks 4 and 8. Patients continued to follow the low-fat diet and received diet counseling throughout the study.

The patients were randomly assigned one of three treatments according to a computer-generated randomization code issued by the central coordinating center. In treatment group 1, patients received 10 mg ezetimibe daily plus placebo for 12 weeks. In treatment group 2, patients received 5.0 mg AEGR-733 for the first 4 weeks, 7.5 mg for the second 4 weeks, and 10 mg for the last 4 weeks, plus placebo for 12 weeks. In treatment group 3 patients received AEGR-733 (with the same dosing schedule as group 2) plus 10 mg ezetimibe daily for 12 weeks. The placebos were identical in appearance to either the ezetimibe tablets or the AEGR-733 tablets,

dependent on which they replaced. The patients were instructed to take the study medication in the morning with breakfast. Patient randomization was not stratified by baseline characteristics because the small sample size in this study would have made such stratification difficult.

Study visit data

During the study visits at 4, 8 and 12 weeks, data were collected from history, physical examinations, electrocardiograms, concomitant medications and on assessment of study drug adherence, which was done by conducting pill counts on the returned drug supply from the patients. Blood samples for laboratory analyses were obtained after a 12 h fast. Plasma was separated from samples, immediately frozen at –20°C, and shipped to the core laboratory on dry ice.

Laboratory assays

Total cholesterol, HDL cholesterol, and triglyceride levels were measured enzymatically on an autoanalyzer (Cobas Fara II, Roche Diagnostic Systems, Basel, Switzerland). Levels of apoB and apolipoprotein A-I (apoA-I) were measured by immunonephelometry on a BNII analyzer (Dade Behring, Brussels, Belgium), and lipoprotein (a) levels were measured by immunoturbidity.

Tracking and recording of adverse events

In addition to the collection of all clinical adverse events, patients also completed the previously validated Gastrointestinal Symptom Rating Scale,[15,16] which consists of five symptom clusters: reflux, abdominal pain, constipation, diarrhea and indigestion. The scale ranges from 1 to 7 (least to most severe symptoms).[17]

Liver function tests were done at each study visit. In any patient who experienced an increase in transaminase levels to more than three times the upper limit of normal on two consecutive occasions the study drug was discontinued, and participants were followed up until transaminase levels returned to baseline. These patients did not enter the study again.

Data analyses

The primary data analyses were performed by an independent statistician employed by the Data Coordinating Center (PharmaNet, Princeton, NJ). The investigators had complete access to the primary data and the data analyses. Normally distributed continuous variables are reported as mean ± SD, and categorical variables as counts and percentages. Differences in continuous variables between treatment groups were assessed by analysis of variance (ANOVA) and *t*-tests. Within-group differences were assessed with paired *t*-tests. Differences in categorical variables between treatment groups were assessed by χ^2 tests. All reported *P* values are two-tailed. All patients who received at least one dose of study drug or placebo in any group were included in the analyses of drug safety and tolerability.

The efficacy analyses included all randomized patients who completed the study. The primary outcome of the study was percentage change in LDL cholesterol from baseline after each of the 4-week treatment periods. This time frame was based on expected maximum effects of ezetimibe within 4 weeks[18,19] and the known LDL-cholesterol-lowering effects of MTP inhibitors.[14] Secondary outcomes were percentage changes in other serum lipoproteins (total cholesterol, non-HDL, VLDL, triglycerides, HDL cholesterol, lipoprotein (a), apoB and apoA-I), change in body weight and overall safety and tolerability.

Sample size estimates

The main comparison used for the sample size was 10 mg ezetimibe alone versus 10 mg AEGR-733 in combination with 10 mg ezetimibe. We estimated that ezetimibe alone would produce an ~18% decrease in LDL-cholesterol.[19] Based on data from an earlier unpublished phase I study; we also estimated that the combination with AEGR-733 would produce an additional 20% decrease in LDL-cholesterol. We calculated, therefore, that an enrollment target of 25 patients per group with a 20% dropout rate would yield 90% power (SD 19%). Significance was set at $P = 0.05$.

RESULTS

Patients

A total of 85 patients were enrolled and randomized (28–29 in each treatment group). The baseline characteristics of these patients are summarized in Table 1. Sixty-seven patients completed the study, 17 discontinued therapy completely owing to adverse events and 1 was lost to follow-up before final efficacy data were obtained (Figure 1).

Effect of AEGR-733 on apolipoprotein B levels

Patients assigned to the combination of ezetimibe plus AEGR-733 experienced dose-dependent reductions in LDL ranging from 35% to 46%

Table 1 Baseline characteristics of all randomized patients.

Characteristic	Ezetimibe ($n=29$)	AEGR-733 (10 mg) ($n=28$)	AEGR-733 (10 mg) plus ezetimibe ($n=28$)
Mean age (years)	54.7 ± 9.0	57.5 ± 7.2	55.1 ± 5.7
Sex (women, %)	62.1	46.4	50.0
Race (white, %)	75.9	78.6	64.3
Mean (SD) BMI (kg/m^2)	28.6 ± 5.4	29.6 ± 5.4	29.6 ± 7
Mean (SD) baseline total cholesterol level (mmol/l)[a]	6.3 ± 0.8	6.6 ± 1.0	6.4 ± 0.9
N with CAD risk factors (%)			
Age >45 years (m) or >55 years (f)	18 (64.3)	22 (78.6)	23 (82.1)
Hypertension	8 (28.6)	12 (42.9)	10 (35.7)
Smoking	10 (35.7)	4 (14.3)	8 (28.6)
Family history of CHD	6 (21.4)	7 (25.0)	9 (32.1)
HDL-cholesterol level <40 mmol/l[a]	2 (7.2)	3 (10.7)	1 (3.6)
N with CAD (%)	0	1 (3.6)	0

[a]To convert to mg/dl divide by 0.0259. Abbreviations: CAD, coronary artery disease; CHD, coronary heart disease; f, female; m, male; *N*, number of patients.

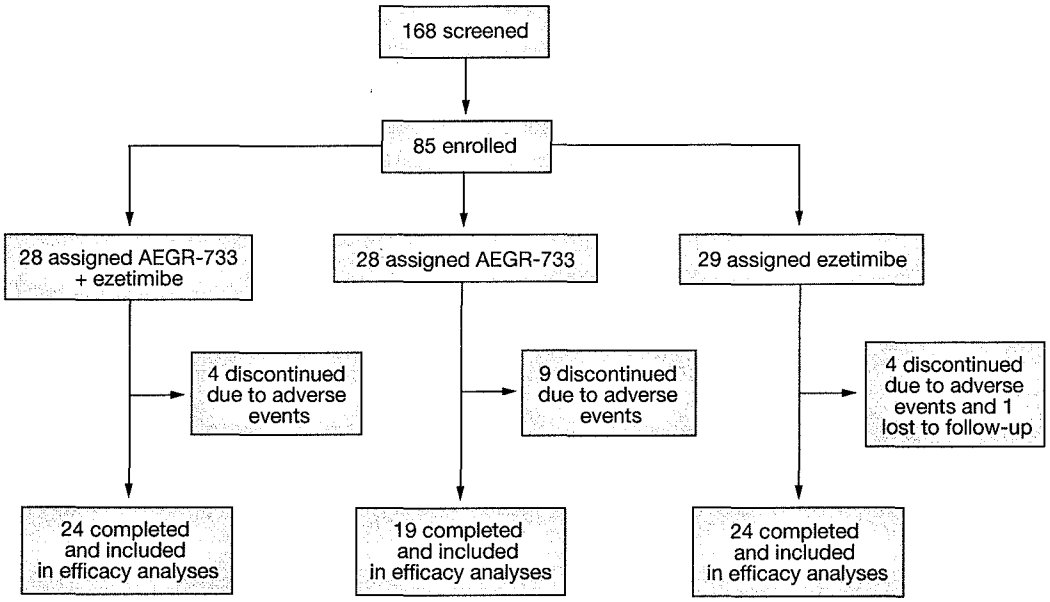

Figure 1 Study profile.

(Figure 2 and Table 2; $P<0.001$ versus ezetimibe alone). Patients assigned ezetimibe monotherapy experienced a 20–22% decrease in LDL-cholesterol levels after 12 weeks of therapy (Figure 2 and Table 2). Patients assigned to AEGR-733 monotherapy experienced dose-dependent reductions in LDL-cholesterol concentrations ranging from 19% to 30% (Figure 2 and Table 2; $P=0.013$ for a greater LDL reduction with 10 mg AEGR-733 alone versus 10 mg ezetimibe alone). Patients receiving AEGR-733 monotherapy also experienced dose-dependent decreases in concentrations of total cholesterol (23% at 10 mg), non-HDL cholesterol (27% at 10 mg) and apoB (24% at 10 mg); these reductions were all greater than those observed with ezetimibe monotherapy (Table 3). Further reductions in total cholesterol, non-HDL cholesterol, and apoB levels were observed in the group receiving combination therapy (Table 3). Triglycerides did not change significantly from baseline in any of the three groups (Table 3). Patients receiving AEGR-733

either alone or in combination with ezetimibe experienced a significant decrease in lipoprotein (a) compared with those receiving ezetimibe alone (Table 3).

Effect of AEGR-733 on apolipoprotein A levels

Patients receiving AEGR-733, alone or with ezetimibe, experienced decreases of 7% or more in HDL-cholesterol levels, which were significantly different from the 6% increase observed with ezetimibe monotherapy ($P<0.001$ for each between-group difference; Table 3). Similar changes in apoA-I were seen (Table 3).

Changes in weight

After 12 weeks, patients assigned ezetimibe monotherapy experienced a mean weight loss of 0.2 ± 1.9 kg (0.1%); those assigned AEGR-733 monotherapy experienced a mean weight loss of 0.7 ± 2.0 kg (1.0%); and those assigned combined AEGR-733 plus ezetimibe experienced a mean weight loss of 1.4 ± 2.6 kg (1.4%); only the latter change was significant ($P=0.013$). However, the weight loss was not significantly different in the combination group from that for the group receiving ezetimibe alone.

Safety

Of the 85 patients enrolled, 18 (20%) either stopped or were taken off study medication before completion of the study (Table 4), mainly owing to mildly elevated transaminase levels. This adverse event occurred in 9 of 56 (18%) patients who took AEGR-733, either alone or in combination with ezetimibe, compared with none of the 29 patients assigned to ezetimibe alone. Transaminase levels returned to baseline in all these patients over the course of the protocol-specified, 2-week follow-up. One patient in the combined AEGR-733 plus ezetimibe group, and two patients in each of the AEGR-733-only and ezetimibe-only groups, dropped out of the study because of gastrointestinal side effects (Table 4). The adverse effects were mild (mean gastrointestinal symptom rating scores ≤2). Patients receiving AEGR-733 alone experienced slightly more gastrointestinal symptoms, and the severity was greater than in the other groups only for constipation ($P=0.007$; Table 4).

DISCUSSION

In this prospective, randomized trial AEGR-733 provided a dose-dependent reduction in LDL cholesterol, reaching a 30% reduction with 10 mg daily monotherapy and a 46% reduction when combined with 10 mg ezetimibe daily. Both of these regimens provided significantly greater lowering effects than ezetimibe monotherapy. Concentrations of other apo-B-containing lipoproteins, including total cholesterol, non-HDL cholesterol and lipoprotein (a), were similarly reduced by AEGR-733.

Patients receiving AEGR-733 experienced a 5–9% reduction in HDL-cholesterol levels, with corresponding reductions in apoA-I. This effect is similar to that observed in two prior studies of MTP-inhibitors.[14,20] In a study of patients with homozygous familial hypercholesterolemia AEGR-733 (studied as BMS-201038) administered at higher doses than used

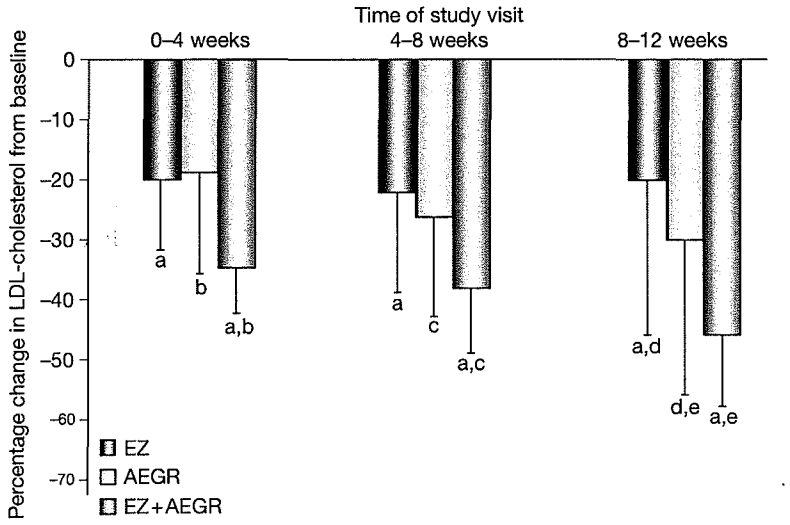

Figure 2 Percentage change from baseline in LDL-cholesterol levels at each of the study visits, by treatment group. Error bars represent SD of the mean. [a]EZ versus EZ+AEGR, $P<0.001$. [b]AEGR versus EZ+AEGR, $P<0.001$. [c]AEGR versus EZ+AEGR, $P=0.015$. [d]EZ versus AEGR, $P=0.016$. [e]AEGR versus EZ+AEGR, $P=0.013$. Abbreviations: AEGR, AEGR-733; EZ, ezetimibe.

Table 2 Changes in LDL-cholesterol levels after 12 weeks of therapy.

Mean (SD) LDL-cholesterol	Mean (SD) values		
	EZ	AEGR-733 (10 mg)	AEGR-733 (10 mg) + EZ
Baseline value (mmol/l)[a]	4.2 ± 0.7	4.4 ± 0.9	4.4 ± 0.7
12-week value (mmol/l)[a]	3.3 ± 0.5	3.1 ± 0.9	2.3 ± 1.1
Percentage change (%)	20 ± 10	30 ± 15[b]	46 ± 24[c,d]

[a]To convert to mg/dl divide by 0.0259. [b]$P=0.015$ for AEGR-733 alone versus ezetimibe alone. [c]$P=0.013$ for AEGR-733 plus ezetimibe versus AEGR-733 alone. [d]$P<0.001$ for AEGR-733 plus ezetimibe versus ezetimibe alone.

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