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Zerlasiran—A Small-Interfering RNA Targeting Lipoprotein(a)
A Phase 2 Randomized Clinical Trial

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IMPORTANCE Elevated lipoprotein(a) increases the risk of atherosclerotic cardiovascular disease (ASCVD) and aortic stenosis.

OBJECTIVE To evaluate the effects of zerlasiran, a small-interfering RNA targeting hepatic synthesis of apolipoprotein(a), on lipoprotein(a) serum concentration.

DESIGN, SETTING, AND PARTICIPANTS A multicenter trial in patients with stable ASCVD with serum lipoprotein(a) concentrations greater than or equal to 125 nmol/L at 26 sites in Europe and South Africa between January 3, 2023, and April 27, 2023, with last follow-up on July 1, 2024.

INTERVENTIONS Participants randomized to receive a subcutaneous dose of placebo every 16 weeks for 3 doses (n = 23) or every 24 weeks for 2 doses (n = 24) or zerlasiran 450 mg every 24 weeks for 2 doses (n = 45), 300 mg every 16 weeks for 3 doses (n = 42), or 300 mg every 24 weeks for 2 doses (n = 44).

MAIN OUTCOME AND MEASURES The primary outcome was the time-averaged percent change in lipoprotein(a) concentration from baseline to 36 weeks, with follow-up to 60 weeks.

RESULTS Among 178 patients, mean (SD) age was 63.7 (9.4) years, 46 (25.8%) were female, with a median (IQR) baseline lipoprotein(a) concentration of 213 (177-282) nmol/L; 172 patients completed the trial. Compared with the pooled placebo group, the least-squares mean time-averaged percent change in lipoprotein(a) concentration from baseline to week 36 was −85.6% (95% CI, −90.9% to −80.3%), −82.8% (95% CI, −88.2% to −77.4%), and −81.3% (95% CI, −86.7% to −76.0%) for the 450 mg every 24 weeks, 300 mg every 16 weeks, and 300 mg every 24 weeks groups, respectively. Median (IQR) percent change in lipoprotein(a) concentration at week 36 was −94.5% (−97.3% to −84.2%) for the 450 mg every 24 weeks group, −96.4% (−97.7% to −92.3%) for the 300 mg every 16 weeks group, and −90.0% (−93.7% to −81.3%) for the 300 mg every 24 weeks group. The most common treatment-related adverse effects were injection site reactions, with mild pain occurring in 2.3% to 7.1% of participants in the first day following drug administration. There were 20 serious adverse events in 17 patients, none considered related to the study drug.

CONCLUSIONS Zerlasiran was well-tolerated and reduced time-averaged lipoprotein(a) concentration by more than 80% during 36 weeks of treatment in patients with ASCVD.

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Visual Abstract

Multimedia

Supplemental content

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Lipoprotein(a) is increasingly recognized as a likely causal risk factor for atherosclerotic cardiovascular disease (ASCVD) and aortic stenosis.[1] Lipoprotein(a) has 2 components, apolipoprotein B_{100} covalently bound to a glycoprotein, apolipoprotein(a).[2] Circulating lipoprotein(a) concentrations are largely determined by the *LPA* gene, which produces messenger RNA (mRNA) that induces hepatic production of apolipoprotein(a), a rate-limiting step in the synthesis of lipoprotein(a). Concentrations of lipoprotein(a) exceed the upper limits of normal in most laboratories (>75 nmol/L) in approximately 25% of the population.[3,4] Lipoprotein(a) concentrations are not reduced by the traditional interventions used to prevent ASCVD, including diet, exercise, and statin medications, although proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors moderately reduce levels. Plasma apheresis has been approved by regulatory authorities to lower lipoprotein(a), but no pharmacological therapies have been approved.

Because lipoprotein(a) levels are principally genetically determined, most current strategies for reducing circulating concentrations consist of nucleic acid-based therapies, either an antisense oligonucleotide or a small-interfering RNA (siRNA) designed to degrade hepatic mRNA responsible for the production of apolipoprotein(a), thereby preventing assembly of lipoprotein(a) particles.[5-9] A drug that blocks the binding of apolipoprotein(a) to apolipoprotein B_{100} is also under development.[10] Determining the optimal dosing strategy for siRNA therapies is challenging because these agents have a long duration of effect, typically ranging from weeks to months. Achieving steady-state reductions in lipoprotein(a) requires a longer time than therapies with shorter pharmacodynamic effects. The current 60-week study was designed to help determine the optimal zerlasiran dose and dosing interval for future phase 3 trials and further assess safety in patients with ASCVD, the target population for this treatment.

Key Points

Question What is the effect of zerlasiran on time-averaged lipoprotein(a) serum concentrations during 36 weeks of treatment?

Findings In 178 patients with cardiovascular disease and lipoprotein(a) concentrations greater than or equal to 125 nmol/L, the least-squares mean placebo-adjusted time-averaged percent change in lipoprotein(a) serum concentrations was −85.6%, −82.8%, and −81.3% for the 450 mg every 24 weeks, 300 mg every 16 weeks, and 300 mg every 24 weeks groups, respectively. The most common adverse events were mild injection site reactions.

Meaning Zerlasiran was well-tolerated and reduced time-averaged lipoprotein(a) concentration by more than 80% during 36 weeks of treatment.

Methods

Study Organization and Oversight

The current trial, Assessment of Lipoprotein(a) lowering in Cardiovascular Disease with SLN360 (ALPACAR-360), is a phase 2 study of zerlasiran, an N-acetylgalactosamine-conjugated siRNA previously evaluated in a phase 1 trial.[7,9] The trial was managed by a contract research organization (Medpace), the sponsor (Silence Therapeutics), and the Cleveland Clinic Coordinating Center for Clinical Research (C5Research). This study was conducted in accordance with the Declaration of Helsinki and international ethical guidelines. The trial protocol was approved by ethics committees or institutional review boards at each site and written informed consent obtained from all participants. A safety review committee monitored the trial. The trial database was transferred to C5Research for statistical analysis following completion of the trial.

Study Population

The trial enrolled adults aged 18 to 80 years with ASCVD defined as a previous myocardial infarction, stroke, angiographically documented coronary artery disease or peripheral arterial disease, or presence of any coronary calcium by computed tomography. Participants undergoing lipid-modifying therapy needed to be following a stable regimen for a minimum of 8 weeks. Patients were excluded for an acute cardiovascular event within the previous 12 weeks, an estimated glomerular filtration rate less than 30 mL/min/1.73 m^2, history of liver disease, or moderate to severe heart failure. Full inclusion and exclusion criteria are described in the trial protocol (Supplement 1). The statistical analysis plan is available in Supplement 2.

Study Procedures

This randomized, double-blind, placebo-controlled trial enrolled patients in 5 groups in a 1:1:2:2:2 ratio to placebo every 16 weeks for 3 doses, placebo every 24 weeks for 2 doses, zerlasiran 450 mg every 24 weeks for 2 doses, zerlasiran 300 mg every 16 weeks for 3 doses, or zerlasiran 300 mg every 24 weeks for 2 doses. Each dose of zerlasiran or placebo was injected subcutaneously in the abdomen at a single site for all doses except the 450-mg dose, which was administered as a split dose at 2 sites. The placebo consisted of 0.9% sodium chloride, which was visually indistinguishable from zerlasiran. The investigators, study participants, and sponsor were blinded to study drug assignment, but delegated site staff (eg, the site pharmacist) were not blinded.

After screening and baseline visits, follow-up visits were scheduled every 4 weeks up to 40 weeks, then at weeks 48 and 60 following randomization. At each postrandomization visit, adverse events were recorded, including injection site reactions, which were assessed for pain, tenderness, erythema, and induration. Lipoprotein(a) serum concentrations and safety laboratory chemistries were obtained at follow-up visits and analyzed in a central laboratory. Lipoprotein(a) serum concentration was measured with a particle-enhanced turbidimetric assay on a Roche c502 autoanalyzer.

Study End Points

The primary efficacy outcome was time-averaged percent change in serum lipoprotein(a) concentration for each zerlasiran treatment group compared with placebo from baseline

through 36 weeks following administration. Time-averaged percent changes from baseline to weeks 48 and 60 were specified as secondary end points. Additional secondary end points included time-averaged percent changes in other lipid parameters (low-density lipoprotein cholesterol [LDL-C], apolipoprotein B, high-density lipoprotein cholesterol [HDL-C], total cholesterol, and triglycerides) from baseline to weeks 36, 48, and 60. Percent and absolute changes (not time-averaged) in lipoprotein(a) were also prespecified secondary end points. The safety and tolerability of zerlasiran during 60-week follow-up were designated as secondary end points, with adverse events coded by system organ class and preferred term using the Medical Dictionary for Regulatory Activities. Safety laboratory studies were prespecified as secondary end points, including the number and percentage of patients with liver enzyme abnormalities.

Sample Size

Five treatment groups with approximately 160 participants total were planned, with 20 participants in each placebo group and 40 participants in each zerlasiran treatment group. Based on the phase 1 single- and multi-dose trials, the minimum assumed effect size for the primary end point was a 60% time-averaged reduction in lipoprotein(a) with a pooled SD of 40%.[7,9] Ten participants per treatment group would provide 90% power to detect a 60% difference in time-averaged lipoprotein(a) at the 5% significance level ($P < .05$). A larger sample size than required by the power calculations was chosen to provide greater clarity on the safety and tolerability of the studied doses.

Statistical Analysis

The primary estimand was the time-averaged percent change in lipoprotein(a) from baseline to week 36. Participants assigned to the 2 placebo treatment groups were pooled to create a common placebo group. Analysis of variance was used to test for differences between each active treatment group and the pooled placebo group for time-averaged percent change in lipoprotein(a). The least-squares means, standard errors, and 2-sided 95% CIs for each treatment group were specified for the pairwise comparisons between each zerlasiran treatment group and the pooled placebo group. Pairwise comparisons between zerlasiran treatment groups were considered exploratory and are not included in this manuscript.

The Holm procedure was used to control multiplicity for pairwise comparisons between each zerlasiran group and the pooled placebo group, with *P* values ordered from the smallest to the largest.[11] The smallest *P* value was assigned an α of .05/3 or .0167, the next-smallest *P* value was assigned an α b of .05/2 or .025, and the third-smallest *P* value was assigned an α of .05.

Descriptive statistics were used to summarize safety end points for each treatment group. For categorical variables, summary tabulations of frequency and percentage of subjects within each category were assessed. For continuous variables, the number of participants and mean and SD or median and IQR are presented by treatment group. The protocol in Supplement 1 and statistical analysis plan in Supplement 2 provide full details of the analyses, which were performed using SAS version 9.4 (SAS Institute).

Results

Study Population and Disposition

The trial participants were enrolled between January 3, 2023, and April 27, 2023, with last follow-up on July 1, 2024, at 26 research sites in Australia, the Czech Republic, Denmark, the Netherlands, South Africa, Slovakia, and the United Kingdom. The trial randomized 180 patients, with 2 not treated due to ineligibility and 172 patients completing the study. **Figure 1** shows the flow of participants through the trial.

Table 1 reports the baseline characteristics of the participants. Enrollment included 46 female (26%) and 132 male (74%) participants (mean [SD] age, 63.7 [9.4] years) with a median (IQR) baseline lipoprotein(a) concentration of 213 (177-282) nmol/L. Self-identified race included 8 Asian, 3 Black, 153 White, and 14 participants who reported multiracial or mixed ancestries. Race was collected because lipoprotein(a) concentration is known to vary among different racial and ethnic groups. Median (IQR) LDL-C was 63.5 (47.0-86.0) mg/dL and median apolipoprotein B was 69.5 (61.0-85.0) mg/dL. Cardiovascular disease history included a prior myocardial infarction in 92 (51.7%), peripheral arterial disease in 19 (10.6%), an ischemic stroke in 14 (7.9%), and previous coronary revascularization in 122 (68.5%) patients. Baseline characteristics were broadly similar across different treatment groups.

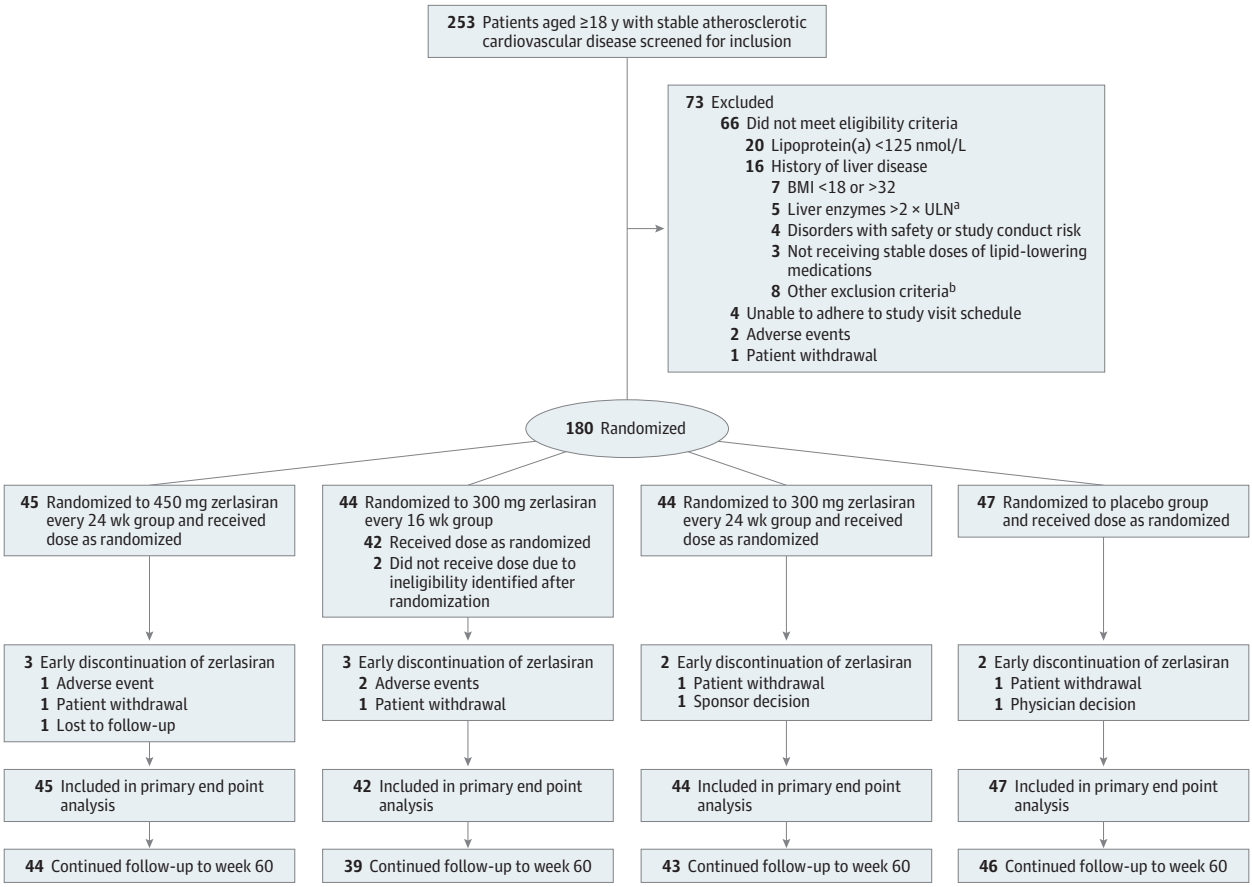
Primary Efficacy End Point

Results for the primary end point are reported in **Table 2**. The group that received 450 mg every 24 weeks for 2 doses showed a placebo-adjusted time-averaged percent change from baseline to 36 weeks of −85.6% (95% CI, −90.9% to −80.3%). The treatment group that received 300 mg every 16 weeks for 3 doses showed a mean placebo-adjusted time-averaged percent change from baseline to 36 weeks of −82.8% (95% CI, −88.2% to −77.4%) and the group that received 300 mg every 24 weeks for 2 doses showed a time-averaged difference of −81.3% (95% CI, −86.7% to −76.0%). The median (IQR) percent change in lipoprotein(a) concentration at week 36 was −94.5% (−97.3% to −84.2%) for the 450 mg every 24 weeks group and −96.4% (−97.7% to −92.3%) and 90.0% (−93.7% to −81.3%) for the 300 mg every 16 weeks and every 24 weeks groups, respectively. **Figure 2** shows waterfall plots for the percent change in lipoprotein(a) at week 36 for individual patients for each dose group for the primary end point. **Figure 3** and eFigure 1 in Supplement 3 show the percent change from baseline in lipoprotein(a) during 60-week follow-up for the different dose groups.

Secondary Outcomes

Results for secondary outcomes are reported in Table 2 and eTables 1 through 4 in Supplement 3. Secondary outcomes are

Figure 1. Trial Patient Flowchart



[a]Three of 5 patients had Gilbert syndrome.

[b]Unlikely to adhere to study procedures (2), history of malignancy within 5 years (1), estimated glomerular filtration rate of <30 mL/min/1.73 m^2 (1), taking niacin (1), taking herbal medications (1), recreational substance use (1), major exclusionary medical disorder (1).

BMI indicates body mass index (calculated as weight in kilograms divided by height in meters squared); ULN, upper limit of normal.

shown graphically in eFigures 2 through 6 in Supplement 3. For the group administered 450 mg every 24 weeks, placebo-adjusted time-averaged percent change from baseline in lipoprotein(a) concentration during 48- and 60-week follow-up was −83.0% (95% CI, −88.4% to −77.5%) and −77.1% (95% CI, −83.1% to −71.2%), respectively. For the group administered 300 mg every 16 weeks, placebo-adjusted time-averaged percent change from baseline in lipoprotein(a) concentration to 48 and 60 weeks was −83.1% (95% CI, −88.7% to −77.6%) and −79.2% (95% CI, −85.3% to −73.1%), respectively. For the group assigned to 300 mg every 24 weeks, the placebo-adjusted time-averaged percent change from baseline in lipoprotein(a) concentration to 48 and 60 weeks was −78.7% (95% CI, −84.2% to −73.2%) and −71.8% (95% CI, −77.8% to −65.8%), respectively. The median (IQR) percent changes in lipoprotein(a) for each visit are reported in eTable 1 in Supplement 3. The maximum median (IQR) percent reduction in lipoprotein(a) concentration was −97.2% (−98.1% to −94.7%), −96.4% (−97.7% to −92.3%), and −95.7% (−97.0% to −92.8%), for the 450 mg every 24 weeks, 300 mg every 16 weeks, and 300 mg every 24 weeks groups, respectively.

Placebo-adjusted time-averaged percent change from baseline in LDL-C to 36 weeks for the 450 mg every 24 weeks, 300 mg every 16 weeks, and 300 mg every 24 weeks groups was −25.1% (95% CI, −46.9% to −3.3%), −31.9% (95% CI, −54.1% to −9.7%), and −29.7% (95% CI, −51.6% to −7.8%), respectively. The placebo-adjusted time-averaged percent change from baseline for apolipoprotein B during 36, 48, and 60 weeks of follow-up is reported in Table 2. The time-averaged absolute change from baseline for lipoprotein(a), LDL-C, and apolipoprotein B is reported in eTable 3 in Supplement 3. There were no clinically significant differences from placebo between any of the dosing regimens for HDL-C or triglycerides (eTable 4 in Supplement 3).

Adverse Events

Treatment-emergent adverse events (TEAEs) are reported in **Table 3**. The most common TEAEs were headache, nasopharyngitis, and urinary tract infection. Two TEAEs led to

Table 1. Baseline Characteristics of Participants

Characteristic	No. (%)			
	450 mg every 24 wk × 2 doses group[a] (n = 45)	300 mg every 16 wk × 3 doses group[a] (n = 42)	300 mg every 24 wk × 2 doses group[a] (n = 44)	Pooled placebo group[a] (n = 47)
Demographics				
Age, mean (SD), y	63.8 (10.2)	63.1 (9.8)	64.5 (8.7)	63.6 (9.1)
Female	11 (24.4)	11 (26.2)	13 (29.5)	11 (23.4)
Male	34 (75.6)	31 (73.8)	31 (70.5)	36 (76.6)
Individuals of childbearing potential	0	1 (2.4)	0	1 (2.1)
BMI, median (IQR)	26.5 (24.5-28.4)	27.1 (25.1-29.7)	28.2 (23.7-30.2)	26.5 (25.0-28.7)
Race[b]				
Asian	4 (8.9)	1 (2.4)	2 (4.5)	1 (2.1)
Black	2 (4.4)	1 (2.4)	0	0
White	36 (80.0)	38 (90.5)	36 (81.8)	43 (91.5)
Multiracial[c]	3 (6.7)	2 (4.8)	6 (13.6)	3 (6.4)
History of ASCVD[d]				
Prior coronary revascularization	29 (64.4)	29 (69.0)	30 (68.2)	34 (72.3)
Prior myocardial infarction	24 (53.3)	19 (45.2)	24 (54.5)	25 (53.2)
Angiographic coronary disease	20 (44.4)	22 (52.4)	26 (59.1)	24 (51.1)
Peripheral arterial disease	6 (13.3)	3 (7.1)	3 (6.8)	7 (14.9)
Prior ischemic stroke	4 (8.9)	3 (7.1)	4 (9.1)	3 (6.4)
Other medical history[d]				
Hypertension	26 (57.8)	30 (71.4)	29 (65.9)	32 (68.1)
Diabetes	7 (15.6)	8 (19.0)	10 (22.7)	10 (21.3)
Medications				
Statin use	38 (84.4)	38 (90.5)	40 (90.9)	39 (83.0)
PCSK9 inhibitor use	6 (13.3)	3 (7.1)	3 (6.8)	11 (23.4)
Laboratory findings[e]				
Lipoprotein(a), median (IQR), nmol/L	216 (182-337)	221 (181-270)	225 (183-296)	203 (164-242)
Total cholesterol, mean (SD), mg/dL	145 (35)	147 (45)	147 (37)	144 (37)
LDL-C, mean (SD), mg/dL	71 (33)	69 (35)	69 (30)	64 (28)
HDL-C, mean (SD), mg/dL	49 (11)	51 (17)	52 (14)	53 (22)
Triglycerides, median (IQR), mg/dL	120 (85-144)	106 (78-193)	125 (85-156)	102 (74-181)
Apolipoprotein B, mean (SD), mg/dL	75 (23)	77 (30)	75 (22)	72 (19)
Oxidized LDL, mean (SD), units/L[f]	47.9 (16.9)	51.4 (16.8)	50.0 (15.9)	48.9 (23.1)
C-reactive protein, median (IQR), mg/L	0.7 (0.4-1.4)	0.7 (0.3-1.5)	0.8 (0.3-2.0)	0.9 (0.3-2.0)

Abbreviations: ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PCSK9, proprotein convertase subtilisin/kexin type 9.

SI conversion factors: to convert total cholesterol, LDL-C, and HDL-C to mmol/L, multiply by 0.0259; triglycerides to mmol/L, multiply by 0.0113; apolipoprotein B to g/L, multiply by 0.01; C-reactive protein to mg/dL, divide by 10.

[a] Unless otherwise indicated.

[b] Race was self-reported by patients and collected because lipoprotein(a) levels are known to vary among racial groups.

[c] Includes participants who self-reported as multiracial or mixed race.

[d] Participants self-reported medical history, which was verified by either a primary care physician or provided directly by a specialist.

[e] Reference ranges: lipoprotein(a), <75 nmol/L; total cholesterol, 100-200 mg/dL; LDL-C, 50-130 mg/dL; HDL-C, 35-60 mg/dL; triglycerides, 50-150 mg/dL; apolipoprotein B, 55-105 mg/dL; oxidized LDL, 24.6-97.0 U/L; C-reactive protein, 0-3 mg/L.

[f] Oxidized LDL represents modification of both lipid and apolipoprotein B components by lipid peroxidation.

withdrawal from the trial, prostate cancer and disseminated tuberculosis, both occurring in the 300 mg every 16 weeks dose group. The most common treatment-related adverse events were injection site reactions, which were transient and mild in severity, with pain occurring in 2.3% to 7.1% of participants in the first day following drug administration and none leading to withdrawal from the trial or missed dosing (eTable 5 in Supplement 3). Twenty serious TEAEs were reported in 17 patients, including 4 in the placebo group, with 3 leading to discontinuation of treatment and none described by investigators as related to the study drug (eTable 6 in Supplement 3). Two patients had single, isolated elevations of liver enzymes, both slightly above 3 times the upper limit of normal with a normal bilirubin, which resolved spontaneously.

Table 2. Primary and Secondary Efficacy End Points[a]

	Least-squares mean (95% CI), %		
	450 mg every 24 wk × 2 doses group	300 mg every 16 wk × 3 doses group	300 mg every 24 wk × 2 doses group
Primary efficacy end point			
Time-averaged percent change from baseline through 36 wk			
Lipoprotein(a)			
Difference from placebo	−85.6 (−90.9 to −80.3)	−82.8 (−88.2 to −77.4)	−81.3 (−86.7 to −76.0)
Secondary efficacy end points			
Time-averaged percent change from baseline through 36 wk			
LDL-C			
Difference from placebo	−25.1 (−46.9 to −3.3)	−31.9 (−54.1 to −9.7)	−29.7 (−51.6 to −7.8)
Apolipoprotein B			
Difference from placebo	−15.0 (−20.1 to −9.8)	−13.3 (−18.6 to −8.1)	−9.9 (−15.0 to −4.7)
Time-averaged percent change from baseline through 48 wk			
Lipoprotein(a)			
Difference from placebo	−83.0 (−88.4 to −77.5)	−83.1 (−88.7 to −77.6)	−78.7 (−84.2 to −73.2)
LDL-C			
Difference from placebo	−26.0 (−44.7 to −7.2)	−29.8 (−48.9 to −10.8)	−27.4 (−46.2 to −8.6)
Apolipoprotein B			
Difference from placebo	−14.0 (−19.2 to −8.8)	−12.4 (−17.6 to −7.1)	−8.6 (−13.9 to −3.4)
Time-averaged percent change from baseline through 60 wk			
Lipoprotein(a)			
Difference from placebo	−77.1 (−83.1 to −71.2)	−79.2 (−85.3 to −73.1)	−71.8 (−77.8 to −65.8)
LDL-C			
Difference from placebo	−24.1 (−43.9 to −4.2)	−28.7 (−48.9 to −8.5)	−26.2 (−46.1 to −6.2)
Apolipoprotein B			
Difference from placebo	−12.6 (−18.0 to −7.2)	−11.3 (−16.8 to −5.7)	−7.2 (−12.6 to −1.7)

Abbreviation: LDL-C, low-density lipoprotein cholesterol.

[a] Time-averaged percent change was estimated by calculating the area under the curve using the linear trapezoidal method.

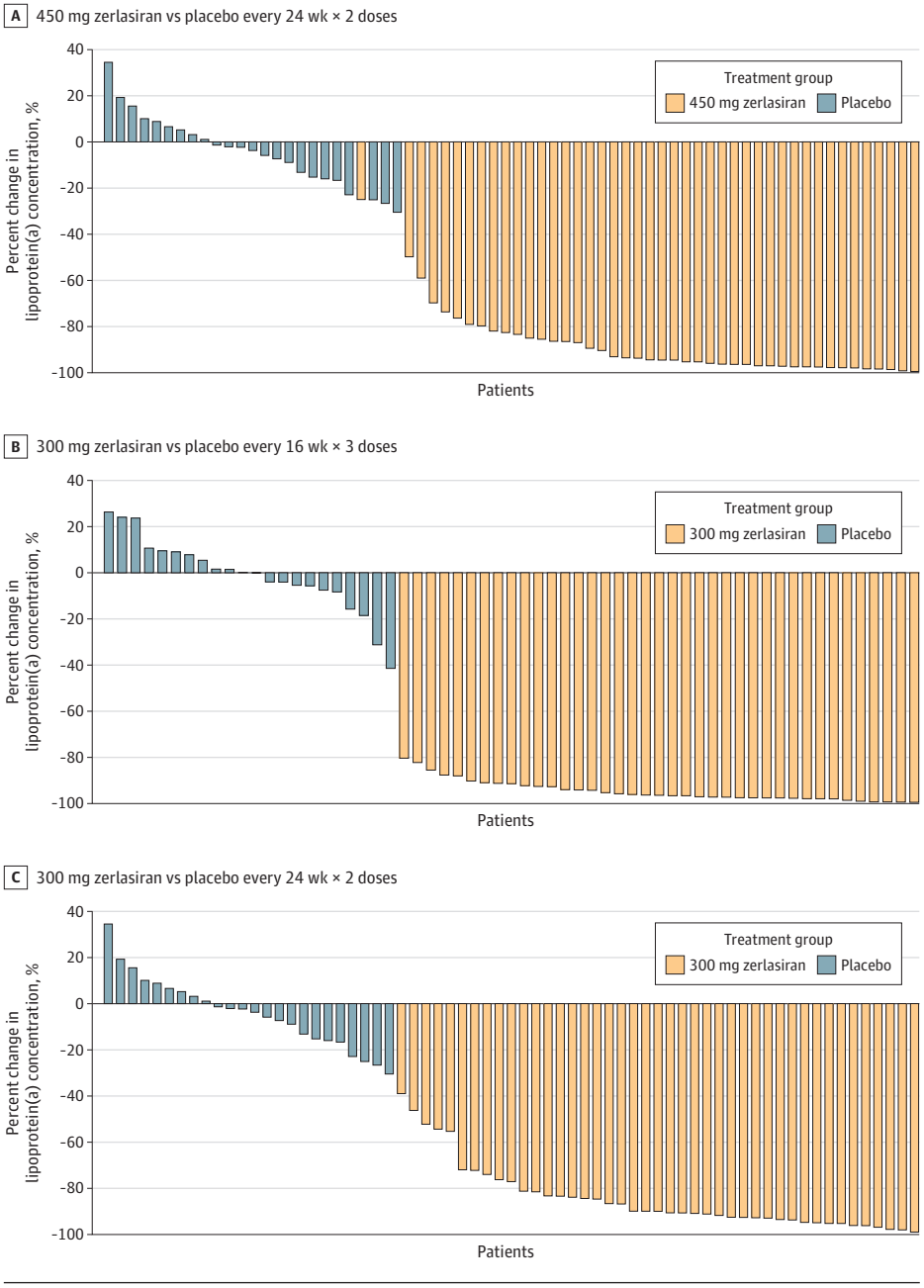
Discussion

In this phase 2 trial of zerlasiran, 3 dosing strategies were studied: 450 mg every 24 weeks for 2 doses, 300 mg every 16 weeks for 3 doses, and 300 mg every 24 weeks for 2 doses. For the primary end point, time-averaged reduction in lipoprotein(a) during 36 weeks of follow-up, all 3 regimens produced an 80% or greater time-averaged reduction compared with placebo. The current end point was different from prior trials of nucleic acid-based therapies, which all used either maximal reduction in lipoprotein(a) or lowering at a specific time point. Time-averaged reduction was prespecified as the primary end point to more accurately describe the effects of treatment over time, including intervals between doses. Maximum median percent reductions ranged from −96.4% to −97.2% for the 3 dose regimens. Waterfall plots showed consistent effects among patients treated with zerlasiran for each of the dose groups. At the final visit 60 weeks following initial drug administration, persistent reductions in lipoprotein(a) were observed, with the largest median reduction, 58.8%, observed for the 300 mg every 16 weeks regimen. Safety data showed no serious adverse events related to the study drug. Although transient injection site reactions were observed, none led to withdrawal from the study or missed dosing.

Evaluation of results from the current trial will allow selection of doses and dosing intervals for a phase 3 clinical trial. Prior to the current study, uncertainty existed as to whether there would be a cumulative effect on lipoprotein(a) reduction after several doses of an siRNA. The effects of 3 doses of 300 mg administered every 16 weeks did show a greater effect with each subsequent dose. After the first dose, a 66% median reduction in lipoprotein(a) was observed 16 weeks after administration, which increased to 81% at 32 weeks (16 weeks after the second dose) and 83% at 48 weeks (16 weeks after the third dose). Similar effects were observed for both the 300- and 450-mg doses administered every 24 weeks, although only 2 doses were studied, so the effects of a third dose were not available. The cumulative effects on lipoprotein(a) could allow consideration of less frequent dosing in a long-term phase 3 trial where patients will be followed for several years.

The effects of zerlasiran on LDL-C and apolipoprotein B were prespecified secondary end points. Compared with placebo, mean time-averaged reductions in LDL-C from

Figure 2. Waterfall Plots Showing Distribution of Lipoprotein(a) Responses for Dosing Groups



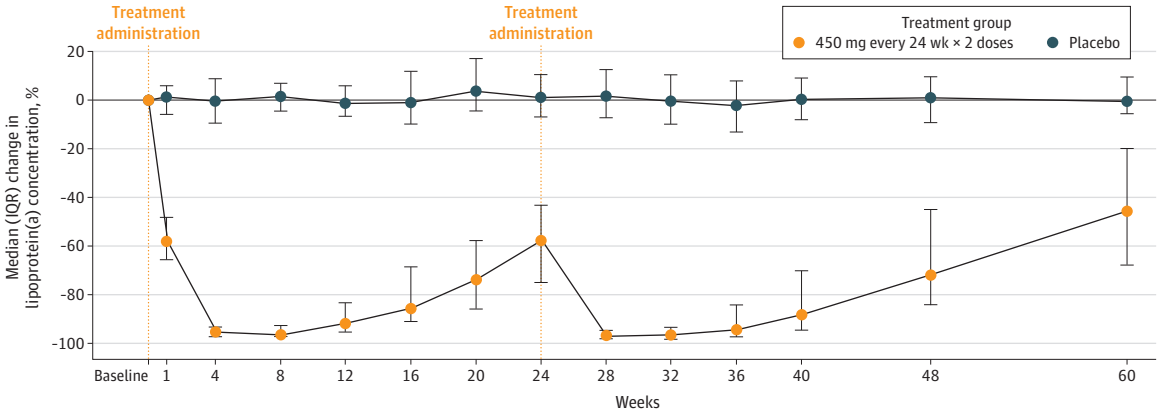
Individual responses for time-averaged percent change in lipoprotein(a) concentration during 36 weeks of follow-up after administration of 2 doses of 450 mg zerlasiran or placebo at a 24-week dosing interval (A). Individual responses for time-averaged percent change in lipoprotein(a) concentration during 36 weeks of follow-up after administration of 3 doses of 300 mg zerlasiran or placebo at a 16-week dosing interval (B). Individual responses for time-averaged percent change in lipoprotein(a) concentration during 36 weeks of follow-up after administration of 2 doses of 300 mg zerlasiran or placebo at a 24-week dosing interval (C).

baseline to 36 weeks ranged from 25.1% to 31.9% and reductions in apolipoprotein B ranged from 9.9% to 15.0%. Other siRNAs targeting lipoprotein(a) have reported a reduction in these atherogenic lipoproteins during treatment.[6] The measurement of LDL-C in patients with elevated lipoprotein(a) is complicated because of the presence of cholesterol content within lipoprotein(a) particles requiring novel methods for quantitation of LDL-C.[12] It remains uncertain whether the observed reduction in conventionally measured LDL-C may contribute to potential benefits on adverse cardiovascular outcomes, although historically, reductions in LDL-C of 15% to 20% have been consistently associated with a reduction in major cardiovascular events.[13]

The relatively long duration of action of siRNA therapeutics is related to the inherent properties of this class of nucleic acid–based drugs.[14] SiRNA therapeutics are small, double-stranded RNAs typically 19 to 25 nucleotides in length. Once the siRNA enters the cell, it is incorporated into an RNA-induced silencing complex, where it unwinds into separate strands. One of these strands, the antisense strand, is complementary to the mRNA that codes for the target protein and facilitates cleavage of mRNA by the Argonaute 2

Figure 3. Percent Changes in Lipoprotein(a) During 60 Weeks of Treatment

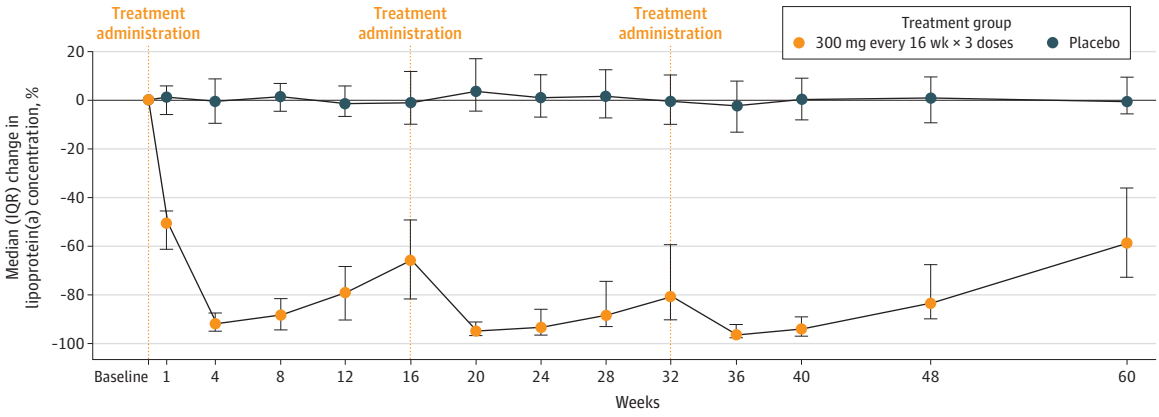
A Percent change in lipoprotein(a) during 60-week follow-up, 450 mg every 24 wk group



No. of patients

450 mg every 24 wk × 2 doses	45	45	45	45	44	44	44	44	42	43	44	44	44	44
Placebo	47	47	46	46	47	46	45	44	45	46	46	45	45	46

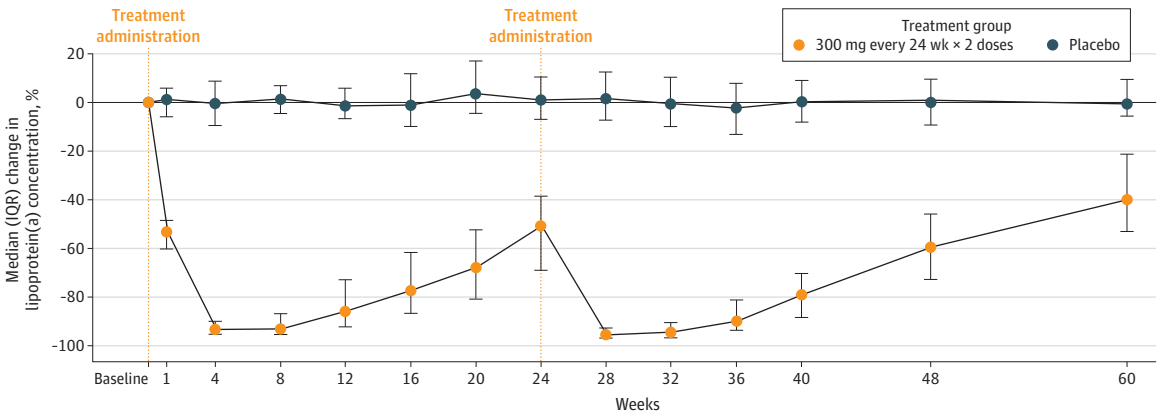
B Percent change in lipoprotein(a) during 60-week follow-up, 300 mg every 16 wk group



No. of patients

300 mg every 16 wk × 3 doses	42	40	42	42	40	40	39	40	40	40	39	39	39	39
Placebo	47	47	46	46	47	46	45	44	45	46	46	45	45	46

C Percent change in lipoprotein(a) during 60-week follow-up, 300 mg every 24 wk group



No. of patients

300 mg every 24 wk × 2 doses	44	44	44	44	43	44	42	42	42	43	43	43	43	43
Placebo	47	47	46	46	47	46	45	44	45	46	46	45	45	46

Markers indicate median percent change in lipoprotein(a) molar concentration and whiskers demonstrate the IQR over time after administration of zerlasiran. Data points for each value used to plot figures reported in eTable 1 in Supplement 3. Absolute changes in lipoprotein(a) reported in eTable 3 in Supplement 3.

Table 3. Adverse Events Reported by Investigators

Characteristic	Dose group during 60-wk follow-up, No. of patients (%) (N = 178)			
	450 mg every 24 wk × 2 doses (n = 45)	300 mg every 16 wk × 3 doses (n = 42)	300 mg every 24 wk × 2 doses (n = 44)	Pooled placebo (n = 47)
Serious adverse events[a]	6 (13.3)	8 (19.0)	2 (4.5)	4 (8.5)
Serious adverse events deemed related to study drug by the investigator	0	0	0	0
Serious adverse events leading to withdrawal from treatment	1 (2.2)	2 (4.8)	0	0
Participants with any treatment-emergent adverse event[b]	42 (93.3)	42 (100.0)	43 (97.7)	39 (83.0)
Participants with any study drug–related treatment-emergent adverse event	36 (80.0)	35 (83.3)	37 (84.1)	8 (17.0)
Classified by maximum severity				
Grade 1 (mild)	22 (48.9)	27 (64.3)	29 (65.9)	7 (14.9)
Grade 2 (moderate)	14 (31.1)	8 (19.0)	8 (18.2)	1 (2.1)
Grade 3 (severe)	0	0	0	0
Grade 4 (life-threatening)	0	0	0	0
Grade 5 (death)	0	0	0	0
Treatment-emergent adverse events occurring in 5% or more of participants in any dose group				
Headache	6 (13.3)	5 (11.9)	3 (6.8)	2 (4.3)
Nasopharyngitis	6 (13.3)	3 (7.1)	6 (13.6)	2 (4.3)
Urinary tract infection	3 (6.7)	5 (11.9)	1 (2.3)	4 (8.5)
Influenza	1 (2.2)	4 (9.5)	3 (6.8)	4 (8.5)
Malaise	4 (8.9)	6 (14.3)	0	1 (2.1)
Myalgia	7 (15.6)	1 (2.4)	1 (2.3)	2 (4.3)
Arthralgia	2 (4.4)	3 (7.1)	4 (9.1)	2 (4.3)
Dizziness	1 (2.2)	3 (7.1)	4 (9.1)	2 (4.3)
Angina pectoris	2 (4.4)	1 (2.4)	1 (2.3)	5 (10.6)
Treatment-emergent adverse events leading to withdrawal from study	0	2 (4.8)	0	0
Safety laboratories				
Liver enzyme elevations[c]	1 (2.2)	1 (2.4)	0	0

Abbreviation: ULN, upper limit of normal.

[a] Adverse events requiring hospitalization (full list in eTable 6 in Supplement 3).

[b] Defined as an event that emerges during treatment, having been absent pretreatment, or worsens relative to the pretreatment state.

[c] Alanine aminotransferase >3 × ULN, aspartate aminotransferase >3 × ULN, alkaline phosphatase >2 × ULN, or total bilirubin >2 × ULN.

protein. Although RNAs are typically rapidly degraded by endonucleases, the antisense strands in therapeutic siRNAs are protected in the RNA-induced silencing complex and chemically modified to resist degradation, thereby allowing a persistence of activity.

Zerlasiran is conjugated with N-acetylgalactosamine, a sugar-like molecule that binds to asialoglycoprotein receptors expressed on the surface of hepatocytes, resulting in rapid endocytosis.[15] The conjugation of siRNAs with N-acetylgalactosamine has been important in the development of these drugs when directed at hepatic targets. This approach reduces residence time in the systemic circulation, which potentially enhances drug safety by avoiding accumulation in nonhepatic tissues. Although evaluating efficacy at different doses is the primary goal of phase 2 trials, safety is always an important end point. In the current trial, adverse effects of concern did not emerge, although the sample size of 178 patients, with 131 receiving zerlasiran, is relatively small and does not exclude more rare adverse events.

Several pharmaceutical agents directed at lipoprotein(a) as a therapeutic target are currently in various stages of development.[5-9] The interest in these therapies arises from the strong genetic and epidemiological data suggesting a relationship between lipoprotein(a) concentration and major cardiovascular events.[16,17] Although no pharmacological therapy has demonstrated effectiveness in improving cardiovascular outcomes, phase 3 trials currently underway are expected to report results within the next several years. Prediction of the magnitude of lipoprotein(a) reduction needed to reduce cardiovascular events has been controversial, with estimates widely varying from 50 mg/dL to greater than 100 mg/dL.[18,19]

Limitations

This study has limitations. First, the trial enrolled predominantly White, male participants. Because Black patients have higher lipoprotein(a) levels compared with White individuals, the effect of zerlasiran in racial and ethnic minority patients needs further study. Second, this phase 2 trial was moderate in size, not large enough to rule out uncommon adverse events. Third, only 2 doses were administered at the 24-week dosing interval. The long-term effects of zerlasiran administered every 24 weeks remains less certain and must be estimated with modeling rather than observed data.

Conclusions

Zerlasiran produced greater than 80% reductions in time-averaged lipoprotein(a) concentration during 36 weeks following administration at doses of 300 mg every 16 weeks or 300 mg or 450 mg every 24 weeks. Persistent reductions in lipoprotein(a) were observed 60 weeks following initial administration. At these doses, no safety concerns emerged with infrequent dosing. These findings support further development of zerlasiran in phase 3 clinical trials.

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