ORIGINAL ARTICLE

Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

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ABSTRACT

BACKGROUND

Angiopoietin-like protein 3 (ANGPTL3) inhibits lipoprotein and endothelial lipases. *ANGPTL3* loss-of-function genetic variants are associated with decreased levels of low-density lipoprotein cholesterol and triglycerides and a decreased lifetime risk of atherosclerotic cardiovascular disease.

METHODS

We conducted an ascending-dose phase 1 trial to assess the safety and efficacy of CTX310, a lipid-nanoparticle–encapsulated clustered regularly interspaced short palindromic repeats–Cas9 endonuclease (CRISPR-Cas9) messenger RNA (mRNA) and guide RNA targeting hepatic *ANGPTL3* to induce a loss-of-function mutation. Adults who had uncontrolled hypercholesterolemia, hypertriglyceridemia, or mixed dyslipidemia and were receiving maximally tolerated lipid-lowering therapy received a single intravenous dose of CTX310 (0.1, 0.3, 0.6, 0.7, or 0.8 mg per kilogram of body weight). The primary end point was adverse events, including dose-limiting toxic effects.

RESULTS

A total of 15 participants received CTX310 and had at least 60 days of follow-up. No dose-limiting toxic effects related to CTX310 occurred. Serious adverse events occurred in two participants (13%): one participant had a spinal disk herniation, and the other died suddenly 179 days after treatment with the 0.1-mg-per-kilogram dose. Infusion-related reactions were reported in three participants (20%), and one participant (7%) who had elevated levels of aminotransferases at baseline had a transient elevation in aminotransferases to between three times and five times as high as those at baseline, peaking on day 4 and returning to baseline by day 14. The mean percent change in ANGPTL3 level was 9.6% (range, –21.8 to 71.2) with the dose of 0.1 mg per kilogram, 9.4% (range, –25.0 to 63.9) with 0.3 mg per kilogram, –32.7% (range, –51.4 to –19.4) with 0.6 mg per kilogram, –79.7% (range, –86.8 to –72.5) with 0.7 mg per kilogram, and –73.2% (range, –89.0 to –66.9) with 0.8 mg per kilogram.

CONCLUSIONS

Editing of *ANGPTL3* was associated with few adverse events and resulted in reductions from baseline in ANGPTL3 levels. (Funded by CRISPR Therapeutics; Australia New Zealand Clinical Trials Registry number, ACTRN12623000809639.)

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A complete list of the trial investigators is provided in the Supplementary Appendix, available at NEJM.org.

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Elevated levels of atherogenic lipoproteins contribute to atherosclerosis and its clinical sequalae.[1,2] A hepatically produced protein, angiopoietin-like 3 (ANGPTL3), inhibits lipoprotein lipase and endothelial lipase with well-established effects on lipid metabolism.[3] Naturally occurring loss-of-function variants in *ANGPTL3* result in lifelong reductions in the levels of serum triglycerides and low-density lipoprotein (LDL) cholesterol and in reduced risk of atherosclerotic cardiovascular disease, with no apparent harmful effects.[4] Pharmacologic inhibition of ANGPTL3 by means of monoclonal antibodies or RNA-targeted therapeutics is associated with clinically significant reductions in atherogenic lipoproteins but requires long-term administration.[5-8] Editing of *ANGPTL3* with clustered regularly interspaced short palindromic repeats–Cas9 endonuclease (CRISPR-Cas9) has the potential to achieve durable genetic modification after a single treatment and to result in permanent reductions in circulating levels of atherogenic lipoproteins. CTX310 is an investigational lipid-nanoparticle–encapsulated formulation of CRISPR-Cas9 components for in vivo gene editing of the target gene, *ANGPTL3*, to induce a loss-of-function mutation in hepatocytes. In this trial, we assessed the safety, side-effect profile, and efficacy of single ascending doses of CTX310.

Methods

Trial Design and Oversight

The trial was designed and funded by CRISPR Therapeutics and conducted in accordance with the principles of the Declaration of Helsinki and the Good Clinical Practice guidelines of the International Council for Harmonisation. The trial protocol is available with the full text of this article at NEJM.org. A central review board and site-specific ethics committees approved the trial design, and all the participants provided written informed consent. A safety review committee provided oversight of participant safety and approved dose escalation. An independent data-monitoring committee provided additional safety oversight.

The data were collected by PPD, a contract research organization. CRISPR Therapeutics performed the initial statistical analysis. The trial data were transferred to statisticians at the Cleveland Clinic Coordinating Center for Clinical Research, who independently confirmed all results. The first and last authors wrote the initial draft of the manuscript, which was reviewed and approved by all the authors. The sponsor reviewed the manuscript and provided suggested revisions, but final decisions on content were reserved for the academic authors, with no restrictions on the right to publish. The first and last authors vouch for the accuracy and completeness of the data and the fidelity of the trial to the protocol and the statistical analysis plan.

Drug Product

CTX310 consists of two components: messenger RNA (mRNA) encoding *Streptococcus pyogenes* Cas9 and a single guide RNA (sgRNA) targeting the gene of interest, encapsulated together in a lipid nanoparticle. The polyadenylated *S. pyogenes* Cas9 mRNA contains methylated pseudouridine to reduce inflammatory response. The sgRNA is a 100-mer single-stranded oligonucleotide. The lipid nanoparticle is composed of four components: an ionizable lipid, a polyethylene glycol lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, and cholesterol. Lipid nanoparticles undergo receptor-mediated endocytosis in hepatocytes by means of apolipoprotein E–mediated LDL receptor uptake, scavenger receptor–mediated uptake, and potentially other receptors. Endosomal escape delivers the drug product into the cytoplasm with importin-mediated delivery to the nucleus. In preclinical studies of CTX310, off-target editing was not observed. Additional information is provided in the Supplementary Appendix, available at NEJM.org.

Trial Population and Procedures

In this phase 1A, multicenter, open-label trial of a single ascending dose, participants were enrolled at six sites in Australia, New Zealand, and the United Kingdom. Adults who were 18 to 75 years of age were eligible if they had a diagnosis of uncontrolled hypercholesterolemia (familial or nonfamilial), moderate-to-severe hypertriglyceridemia, or mixed dyslipidemia. Inclusion criteria required that the participants have disease that was refractory to maximally tolerated doses of lipid-lowering therapies, defined as at least one of the following: a fasting serum triglyceride level greater than 150 mg per deciliter (1.7 mmol per liter), LDL cholesterol level greater than 100 mg per deciliter (2.6 mmol per liter) or 70 mg per deciliter (1.8 mmol per liter) in participants with atherosclerotic cardiovascular disease, apolipoprotein

B level greater than 100 mg per deciliter, or non–high-density lipoprotein (non-HDL) cholesterol level greater than 160 mg per deciliter (4.1 mmol per liter). Women of childbearing potential were excluded, as were persons with familial chylomicronemia syndrome with less than 5% lipoprotein lipase activity and persons with an estimated glomerular filtration rate of less than 60 ml per minute per 1.73 m^2 of body-surface area. Full inclusion and exclusion criteria are described in the protocol.

Participants were initially enrolled into one of four ascending CTX310-dose cohorts (0.1 mg per kilogram, 0.3 mg per kilogram, 0.6 mg per kilogram, or 0.8 mg per kilogram), with doses calculated by the total amount of RNA administered and based on estimated lean body weight.[9] CTX310 was administered as a single intravenous infusion. A cohort that received 0.7 mg per kilogram was added, and the cohort that received 0.8 mg per kilogram was expanded after the first three participants in that cohort had received the CTX310 infusion, with the goal being to better define the dose–response relationship. Before drug infusion, all the participants received premedication with glucocorticoid agents and antihistamines. Participants were directly observed for a minimum of 24 hours after infusion, and safety assessments occurred daily for 3 days after treatment. Weekly follow-up was performed during the first 30 days after treatment, with subsequent visits scheduled at days 60, 90, 180, 270, and 360 (Fig. S1 in the Supplementary Appendix). All the participants completed at least 60 days of follow-up, with additional follow-up ongoing. Escalation of the doses was approved by the safety review committee after a minimum of three participants were treated at each dose level and completed at least 30 days of follow-up.

END POINTS

The primary objective of the trial was the evaluation of the safety and side-effect profile of single ascending doses of CTX310. The primary end point was the incidence of adverse events as assessed by investigators, including dose-limiting toxic effects, which were graded according to the Common Terminology Criteria for Adverse Events (CTCAE), version 5.0, of the National Cancer Institute.[10] Events were classified as grade 1 to grade 5, with a higher grade indicating greater severity. Full definitions of the CTCAE grading scale are provided in the Supplementary Appendix. Dose-limiting toxic effects were defined as the following: an increase in liver aminotransferase levels to CTCAE grade 3 or higher, with increases persisting for more than 14 days after infusion; an increase in bilirubin levels or international normalized ratio to CTCAE grade 3 or higher; any abnormal grade 3 laboratory-assessed result persisting for 7 days or longer; or any abnormal laboratory-assessed result of severity equal to CTCAE grade 4. Secondary safety end points included the frequency and severity of adverse events, including adverse events of special interest (infusion-related reactions, abnormal bleeding, thrombotic events, or hemorrhagic events; increases in aminotransferase levels; allergic or localized reactions; or new malignant condition).

Secondary efficacy end points included percent changes from baseline over time in the levels of LDL cholesterol, triglycerides, apolipoprotein B, HDL cholesterol, and non-HDL cholesterol. The pharmacodynamic secondary end point was the percent change in ANGPTL3 concentration. The pharmacokinetic secondary end points included plasma levels of lipid nanoparticle components. Exploratory end points are described in the Supplementary Appendix.

STATISTICAL ANALYSIS

The data were analyzed descriptively, without formal hypothesis testing. Safety analyses included all the participants who received CTX310. Adverse events were categorized according to preferred terms in the *Medical Dictionary for Regulatory Activities* (MedDRA), version 28.0. Safety data are summarized according to CTX310 dose level and include all available data for the participants. Efficacy analyses in each dose cohort are reported as the mean change and range for all participants at day 60 or day 90 after administration of CTX310. The percent change in ANGPTL3 concentration is reported at 30 days after drug administration. No imputation was performed for missing values.

RESULTS

PARTICIPANTS

From May 2024 through August 2025, at total of 27 persons underwent screening for eligibility; 15 participants were enrolled in the trial and received CTX310 (3 at a dose of 0.1 mg per kilogram, 3 at 0.3 mg per kilogram, 3 at 0.6 mg per kilo-

Table 1. Demographic and Clinical Characteristics of the Participants at Baseline.*

Characteristic	All Participants (N=15)
Median age (range) — yr	53 (31–68)
Male sex — no. (%)	13 (87)
Body-mass index†	31.1±4.9
White race — no. (%)‡	14 (93)
Clinical ASCVD — no. (%)	6 (40)
Familial hypercholesterolemia — no. (%)§	6 (40)
Severe hypertriglyceridemia — no. (%)	2 (13)
Mixed dyslipidemia — no. (%)¶	6 (40)
Nonfamilial hypercholesterolemia — no. (%)§	1 (7)
ANGPTL3 — ng/ml	161.8±58.0
Cholesterol level — mg/dl	
Total cholesterol	246.3±74.7
HDL cholesterol	43.0±13.7
Directly measured LDL cholesterol	154.6±79.2
Non-HDL cholesterol	203.2±73.1
Triglyceride level (IQR) — mg/dl	192.2 (108.9–252.4)
Lipoprotein(a) level (IQR) — nmol/liter	36.3 (20.0–157.6)
Apolipoprotein B — mg/dl	132.1±43.1
Type 2 diabetes mellitus — no. (%)	5 (33)
Background lipid-lowering therapy — no. (%)	
Statin	9 (60)
Ezetimibe	8 (53)
PCSK9 monoclonal antibody	6 (40)
Fibrate	4 (27)
Icosapent ethyl	1 (7)
Apheresis	1 (7)
Evinacumab	1 (7)
Statin intolerance — no. (%)‖	4 (27)

* Plus–minus values are means ±SD. Baseline characteristics are shown for all treated participants. To convert the values for cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129. ANGPTL3 denotes angiopoietin-like protein 3, ASCVD atherosclerotic cardiovascular disease, HDL high-density lipoprotein, LDL low-density lipoprotein, and PCSK9 proprotein convertase subtilisin–kexin type 9.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡ Race was reported by the participants.

§ Six participants had a clinical diagnosis of familial hypercholesterolemia (one homozygous and five heterozygous). All the participants underwent genetic testing, and five of these participants had a genetically confirmed diagnosis of familial hypercholesterolemia (five heterozygous). The participant who had a clinical diagnosis of homozygous familial hypercholesterolemia had a single pathogenic mutation identified on genetic testing. One participant without genetically confirmed familial hypercholesterolemia (clinical diagnosis of heterozygous familial hypercholesterolemia) was enrolled on the basis of a diagnosis of nonfamilial hypercholesterolemia.

¶ Participants with mixed dyslipidemia were those who had concomitant elevations in low-density lipoprotein cholesterol and triglycerides.

‖ Statin intolerance was reported by the participants.

gram, 2 at 0.7 mg per kilogram, and 4 at 0.8 mg per kilogram) (Fig. S2). The median age of the participants was 53 years (range, 31 to 68); 6 participants (40%) had atherosclerotic cardiovascular disease (defined as a history of myocardial infarction, stroke, or arterial revascularization), and 6 (40%) had a clinical diagnosis of familial hypercholesterolemia, including 5 (33%) who had confirmed pathogenic genetic mutations. The mean (±SD) directly measured LDL cholesterol level before treatment was 155±79 mg per deciliter (4.0±2.0 mmol per deciliter), and the median triglyceride level was 192 mg per deciliter (2.2 mmol per liter; interquartile range, 109 to 252 [1.2 to 2.8]). Additional demographic and clinical characteristics of the participants are shown in Table 1.

SAFETY END POINTS

Adverse events are shown in Table 2. No dose-limiting toxic effects or serious adverse events related to CTX310 were observed. Adverse events of special interest included an allergic reaction in one participant who received a dose of 0.3 mg per kilogram; the allergic reaction manifested as a rash on the upper torso that appeared 1 day after infusion and resolved the following day. Three participants had infusion-related reactions (two who received 0.6 mg per kilogram and one who received 0.8 mg per kilogram); all reactions were CTCAE grade 2 in severity. In each case, symptoms began minutes after the start of the infusion and included back pain and nausea. The reactions were managed by pausing the infusion and administering supportive care (i.e., antiemetics) or repeating the administration of premedications and, optionally, administering acetaminophen. All symptoms resolved, and each participant completed the infusion. A participant with elevated aminotransferase levels at baseline had an infusion-related reaction with the 0.8-mg-per-kilogram dose and had a CTCAE grade 2 elevation in aminotransferase levels (between three and five times that of the baseline measurement). Aminotransferase levels reached a peak on day 4 after treatment, were less than twice the baseline value at day 7, and returned to the baseline level by day 14. There were no concomitant elevations in bilirubin or alkaline phosphatase levels or in prothrombin time. Figure S3 shows the changes over time in aminotransferase, alkaline phosphatase, and bilirubin levels in all the participants. One death occurred

in a participant 179 days after administration of a dose of 0.1 mg per kilogram. Additional details are reported in the Supplementary Appendix. Table S1 shows adverse events classified according to MedDRA terms.

SECONDARY EFFICACY END POINTS

Table 3 shows the effect of CTX310 on levels of ANGPTL3 and lipid biomarkers that were prespecified as secondary end points, including baseline values, follow-up values, absolute changes, and percent changes. Background lipid-lowering therapy was unchanged in all the participants through 60 days of follow-up.

The percent changes from baseline over time for the ANGPTL3 and lipid biomarkers, including the last available values that included all the participants in each treatment group, are shown in Figures 1 and 2. The plasma levels of lipid nanoparticle components are shown in Figure S4. An exploratory analysis of changes in apolipoprotein C3 and remnant cholesterol levels is shown in Table S2.

Table 2. Adverse Events.

Adverse Event	All Participants (N=15)
	no. (%)
Death*	1 (7)
Any serious adverse event†	2 (13)
Serious adverse events related to CTX310	0
Any investigator-reported adverse event‡	14 (93)
Grade 1§	4 (27)
Grade 2¶	9 (60)
Grade 3	0
Grade 4	0
Grade 5*	1 (7)
Adverse event of special interest related to CTX310‡‖	4 (27)
Allergic or localized reaction	1 (7)
Infusion-related reaction**	3 (20)
Elevation in level of AST or ALT††	1 (7)

* One death occurred 179 days after administration of a dose of 0.1 mg per kilogram of body weight. Additional details are available in the Supplementary Appendix.

† A serious adverse event (in addition to the one death) occurred in a participant who received CTX310 at a dose of 0.3 mg per kilogram and was hospitalized for a spinal disk herniation 7 months after treatment.

‡ Participants were counted once at the highest-grade adverse event based on Common Terminology Criteria for Adverse Events.

§ Grade 1 adverse events included insomnia in one participant, elevated white-cell count in one, acute kidney injury in one, and cerumen impaction in one. These events occurred in participants who had no adverse events that arose during treatment that were determined to be related to CTX310.

¶ Grade 2 adverse events included muscle aches in one participant (who had no event related to CTX310), head injury in one participant (same participant had a grade 1 event involving fatigue), spinal disk herniation in one (same participant had a grade 2 elevation in white-cell count), headache and toothache in one (same participant had a grade 1 elevation in white-cell count), leg infection in one (who had no event related to CTX310), gastroenteritis in one (same participant had a grade 1 event involving fatigue), and infusion-related reaction in three (one participant had a concomitant elevation in aminotransferase level).

‖ An infusion-related reaction was observed in two participants, infusion-related reaction and aminotransferase elevation in one, and allergic or localized reaction in one.

** A reaction occurred in two participants who received CTX310 at a dose of 0.6 mg per kilogram and in one who received CTX310 at a dose of 0.8 mg per kilogram.

†† An event was defined as an increase in alanine aminotransferase (ALT) or aspartate aminotransferase (AST) to a level that was at least 3 times the upper limit of the normal range or the baseline value (if the baseline ALT level was greater than the upper limit of the normal range at the time of enrollment).

DISCUSSION

This phase 1 trial showed that intravenous CTX310, an in vivo CRISPR-Cas9 gene-editing drug product targeting *ANGPTL3*, can be administered safely in patients who have dyslipidemia that is refractory to lipid-lowering therapy. In this small trial with a follow-up of limited duration, no dose-limiting toxic effects or serious adverse events attributable to CTX310 were observed. Administration of CTX310 resulted in reductions in the target protein, ANGPTL3, with concomitant reductions in levels of atherogenic lipoproteins. The highest dose administered, 0.8 mg per kilogram, produced a mean reduction of 48.9% in LDL cholesterol levels and 55.2% in triglyceride levels in four participants 60 days after treatment.

A total of three participants had infusion-related reactions of short duration, and a single participant had transient elevations of aminotransferase levels with no clinical sequelae. Similar effects have been reported for other lipid-nanoparticle–delivered therapies.[11,12] Longer follow-up in larger patient populations is required to assess late-emerging or low-incidence safety signals. Regulatory pathways for in vivo gene editing are evolving, and the current guidance from the Food and Drug Administration recommends follow-up for up to 15 years after drug administration.[13] Within the context of a potential once-in-a-lifetime therapy, the safety data are deemed to be acceptable for proceeding with larger studies.

ANGPTL3 is produced almost exclusively by the liver and has broad effects on atherogenic

Table 3. ANGPTL3 and Lipid Biomarker Levels, According to CTX310 Dose.*

Variable	0.1 mg/kg (N=3)	0.3 mg/kg (N=3)	0.6 mg/kg (N=3)	0.7 mg/kg (N=2)	0.8 mg/kg (N=4)
Total administered dose (range) — mg	7.1 (5.2 to 9.5)	20.0 (18.2 to 21.8)	41.8 (35.6 to 49.1)	48.3 (45.7 to 50.9)	46.9 (35.6 to 56.4)
ANGPTL3					
Mean baseline level (range) — ng/ml	197.1 (88.3 to 348.8)	162.9 (132.1 to 202.4)	157.8 (134.1 to 190.4)	135.3 (132.7 to 137.9)	150.6 (139.0 to 164.3)
Mean level at 30-day follow-up (range) — ng/ml	262.9 (69.1 to 597.0)	168.6 (137.4 to 216.6)	107.7 (72.3 to 153.4)	27.3 (18.2 to 36.4)	39.7 (18.2 to 50.8)
Mean absolute change (range) — ng/ml	65.8 (–31.6 to 248.2)	5.7 (–50.7 to 84.5)	–50.1 (–76.6 to –36.7)	–108.0 (–119.8 to –96.2)	–110.9 (-–146.1 to –96.4)
Mean percent change (range)	9.6 (–21.8 to 71.2)	9.4 (–25.0 to 63.9)	–32.7 (–51.4 to –19.4)	–79.7 (–86.8 to –72.5)	–73.2 (–89.0 to –66.9)
LDL cholesterol					
Mean baseline level (range) — mg/dl	166.5 (83.1 to 309.4)	149.9 (25.9 to 257.5)	180.2 (121.0 to 256.4)	127.6 (94.0 to 161.3)	143.5 (97.1 to 239.4)
Mean level at follow-up (range) — mg/dl†	164.6 (69.2 to 298.5)	239.4 (210.4 to 268.4)	95.0 (48.0 to 150.0)	106.1 (59.2 to 153.1)	62.4 (32.1 to 104.0)
Mean absolute change (range) — mg/dl	–1.9 (–37.9 to 42.9)	27.5 (10.8 to 44.1)	–85.2 (–208.4 to –13.1)	–21.5 (–34.8 to –8.1)	–81.1 (–207.3 to –18.9)
Mean percent change (range)	4.2 (–35.4 to 51.6)	15.4 (4.2 to 26.5)	–39.2 (–81.3 to –8.1)	–21.0 (–37.0 to –5.0)	–48.9 (–86.6 to –19.5)
Triglycerides					
Mean baseline level (range) — mg/dl	141.4 (83.3 to 184.2)	464.1 (135.5 to 1007.9)	248.0 (192.2 to 299.4)	148.8 (74.4 to 223.2)	371.6 (105.4 to 1073.5)
Mean level at follow-up (range) — mg/dl†	226.1 (76.2 to 374.7)	968.7 (101.0 to 2595.1)	95.4 (60.2 to 116.9)	97.0 (83.3 to 110.7)	101.4 (47.8 to 174.5)
Mean absolute change (range) — mg/dl	84.7 (–7.1 to 190.4)	504.6 (–39.0 to 1587.2)	–152.6 (–190.4 to –132.0)	–51.8 (–112.5 to 8.9)	–270.1 (–899.0 to –45.2)
Mean percent change (range)	46.7 (–8.5 to 103.4)	38.8 (–25.5 to 157.5)	–62.0 (–68.7 to –53.7)	–19.2 (–50.4 to 11.9)	–55.2 (–83.7 to –37.9)

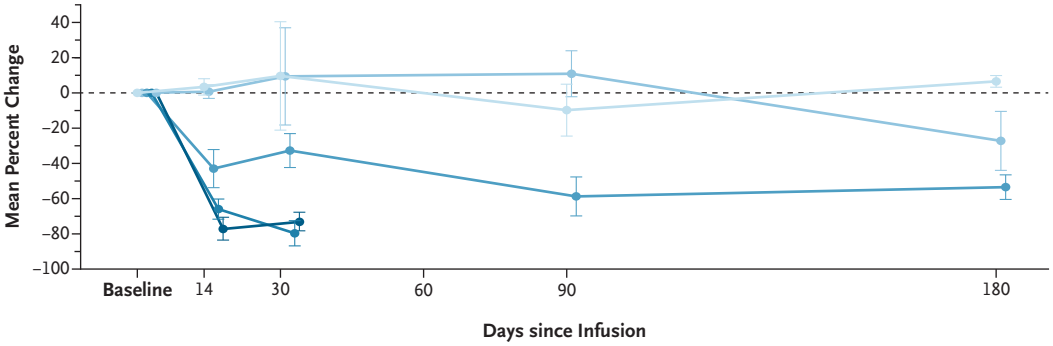
Apolipoprotein B					
Mean baseline level (range) — mg/dl	132.0 (87.0 to 204.0)	139.0 (76.0 to 192.0)	153.7 (109.0 to 211.0)	121.0 (95.0 to 147.0)	116.3 (93.0 to 147.0)
Mean level at follow-up (range) — mg/dl†	144.7 (92.0 to 230.0)	190.5 (180.0 to 201.0)	85.7 (55.0 to 122.0)	95.5 (61.0 to 130.0)	72.0 (38.0 to 93.0)
Mean absolute change (range) — mg/dl	12.7 (–13.0 to 26.0)	20.0 (–12.0 to 52.0)	–68.0 (–156.0 to –19.0)	–25.5 (–34.0 to –17.0)	–44.3 (–109.0 to –4.0)
Mean percent change (range)	9.7 (–12.4 to 28.7)	14.3 (–6.3 to 34.9)	–38.0 (–73.9 to –13.5)	–23.7 (–35.8 to –11.6)	–33.4 (–74.1 to –4.3)
HDL cholesterol					
Mean baseline level (range) — mg/dl	42.4 (39.1 to 45.2)	29.8 (23.2 to 35.2)	38.7 (36.0 to 42.2)	37.5 (37.1 to 37.9)	59.3 (37.9 to 73.1)
Mean level at follow-up (range) — mg/dl†	36.3 (30.9 to 39.1)	30.0 (15.1 to 39.1)	43.7 (37.1 to 51.0)	34.0 (30.2 to 37.9)	45.5 (25.9 to 74.2)
Mean absolute change (range) — mg/dl	–6.1 (–12.0 to 0.0)	0.3 (–8.1 to 5.0)	5.0 (1.2 to 8.9)	–3.5 (–7.0 to 0.0)	–13.7 (–32.1 to 3.1)
Mean percent change (range)	–13.9 (–27.9 to 0.0)	-2.6 (–35.0 to 16.3)	12.5 (3.2 to 21.1)	–9.4 (–18.7 to 0.0)	–24.1 (–43.9 to 4.3)
Non-HDL cholesterol					
Mean baseline level (range) — mg/dl	192.7 (103.2 to 339.5)	234.6 (194.1 to 293.5)	233.3 (171.3 to 316.3)	155.6 (109.0 to 202.0)	188.7 (136.1 to 263.3)
Mean level at follow-up (range) — mg/dl†	206.0 (124.1 to 341.5)	265.4 (253.3 to 285.4)	116.9 (68.1 to 179.4)	125.1 (71.9 to 178.3)	83.4 (52.2 to 113.3)
Mean absolute change (range) — mg/dl	13.3 (–11.2 to 49.1)	30.8 (–8.1 to 63.4)	–116.4 (–248.3 to –32.9)	–30.5 (–37.1 to –24.0)	–105.3 (–211.1 to –34.0)
Mean percent change (range)	13.3 (–8.3 to 47.6)	15.7 (–2.8 to 32.7)	–44.6 (–78.5 to –15.5)	–22.9 (–34.0 to –11.9)	–49.8 (–80.2 to –25.0)

* Doses are in milligrams per kilograms of estimated lean body weight. To convert the values for LDL, HDL, and non-HDL cholesterol to millimoles per liter, multiply by 0.02586. To convert the values for triglycerides to millimoles per liter, multiply by 0.01129.

† Follow-up measurements for lipid biomarkers are reported at 90 days after administration of CTX310 at doses of 0.1 mg per kilogram, 0.3 mg per kilogram, and 0.6 mg per kilogram and at 60 days after doses of 0.7 mg per kilogram and 0.8 mg per kilogram.

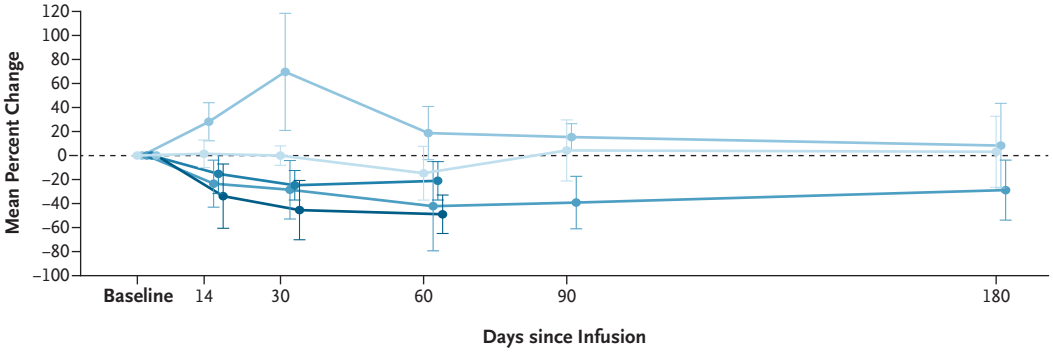
CTX310, 0.1 mg/kg eLBW ● CTX310, 0.3 mg/kg eLBW ● CTX310, 0.6 mg/kg eLBW ● CTX310, 0.7 mg/kg eLBW ● CTX310, 0.8 mg/kg eLBW

A Change in ANGPTL3 Level over Time



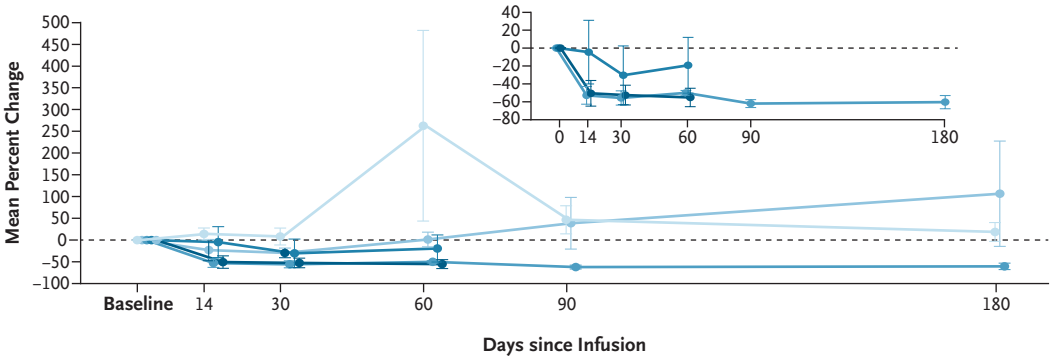
No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.3 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.6 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.7 mg/kg eLBW	2	2	2			
CTX310, 0.8 mg/kg eLBW	4	4	4			

B Change in LDL Cholesterol Level over Time



No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	2	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

C Change in Triglyceride Level over Time



No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

Figure 1 (facing page). Changes in ANGPTL3, LDL Cholesterol, and Triglyceride Levels over Time with CTX310.

Panel A shows the mean percent change in angiopoietin-like protein 3 (ANGPTL3) level from baseline according to visit for each dose level. Panel B shows the mean percent change in low-density lipoprotein (LDL) cholesterol level from baseline according to visit for each dose level. Panel C shows the mean percent change in triglyceride level from baseline according to visit for each dose level. The inset in Panel C shows the results for the three highest dose groups. Day 0 in the inset indicates baseline. The highest dose administered, 0.8 mg per kilogram, produced a mean reduction of 48.9% in LDL cholesterol levels and 55.2% in triglyceride levels in four participants 60 days after treatment. I bars indicate standard errors. For each dose group, the line for percent change extends to the final time point at which data were available for all participants. The abbreviation eLBW denotes estimated lean body weight.

lipoproteins, and therefore it represents an attractive target for gene editing. Lipid nanoparticles are a well-established approach for hepatic delivery of gene-editing therapies.[14] Although many treatments for elevated LDL cholesterol levels exist, current orally administered treatments for hypertriglyceridemia have limited effectiveness, and no treatments simultaneously and substantially lower both LDL cholesterol and triglyceride levels.[15] The observed reductions in LDL cholesterol and triglyceride levels with CTX310 are similar to those observed with evinacumab, a monoclonal antibody targeting ANGPTL3 that has been approved for use in patients with homozygous familial hypercholesterolemia.[5,16,17] Vupanorsen, an antisense oligonucleotide, and two small interfering RNA therapies (zodasiran and solbinsiran) inhibit ANGPTL3 protein synthesis and showed lipid-lowering effects in early-phase trials. Development of vupanorsen was discontinued owing to worsening hepatic steatosis and elevations in aminotransferase levels, whereas zodasiran and solbinsiran are associated with reduced hepatic fat fraction.[6-8]

Although follow-up is ongoing, treatment with CTX310 is aimed at inducing a permanent loss-of-function mutation in *ANGPTL3*. The mechanism of action and reductions in the levels of ANGPTL3 and lipid biomarkers observed with CTX310 through 60 days in participants in the groups receiving the highest doses suggest that extensive gene editing in hepatocytes was probably achieved. In contrast to therapies that require long-term administration, the prospect of one-time therapy may circumvent the well-documented problem of waning adherence to lipid-lowering therapies. Within 12 months after initiation of treatment, up to half of patients discontinue the use of statins and monoclonal antibody proprotein convertase subtilisin–kexin type 9 inhibitors, with adherence decreasing further over subsequent years.[18,19]

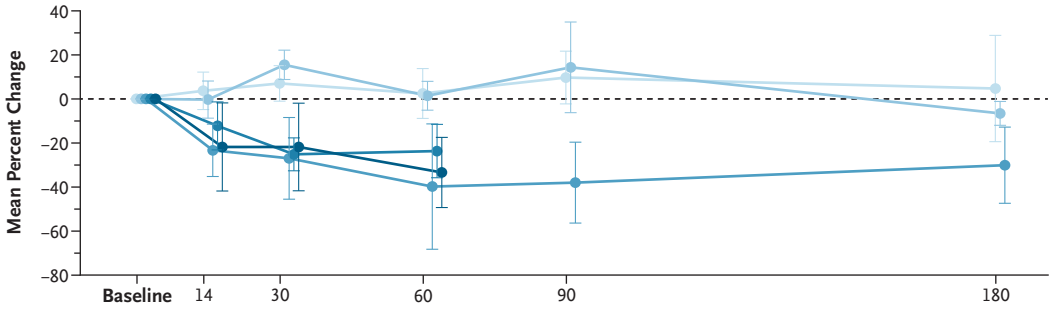
The efficacy of in vivo gene editing and the effect of ANGPTL3 inhibition on atherogenic lipoproteins are probably influenced by factors beyond the administered dose, including hepatic steatosis, inflammation, and preexisting genetic and metabolic profiles.[20,21] This trial population was heterogeneous, with some participants having high levels of LDL cholesterol and others having elevated triglyceride levels. These differences may have contributed to the variability in lipid-lowering effects that was observed among participants who were administered the same CTX310 dose. Further studies are needed to understand patient-specific predictors of treatment effects, editing efficiency, and optimized dose-administration strategies.

In the broader landscape, gene editing with in vivo CRISPR–Cas9 therapy that results in permanent lipid lowering has the potential to add to the therapeutic armamentarium for the reduction of atherogenic lipoproteins. For patients with rare disorders associated with high cardiovascular risk (e.g., homozygous familial hypercholesterolemia) or who have severe hypercholesterolemia from other causes, a single durable intervention could lessen the burden of complex, lifelong medication regimens. For patients with severe hypertriglyceridemia, in whom conventional therapies are often inadequate, a one-time *ANGPTL3* gene-editing treatment is a potentially attractive option. However, as with all irreversible interventions, enthusiasm must be tempered by the need for rigorous, long-term follow-up to ensure both durability and safety.

Our trial has limitations. First, the number of participants was small, and the primary objective of the trial was to establish safety. The small sample size in combination with the open-label design precludes formal statistical comparisons. The enrolled participants had various lipid disorders with broad ranges of baseline values for LDL cholesterol and triglycerides, which limits

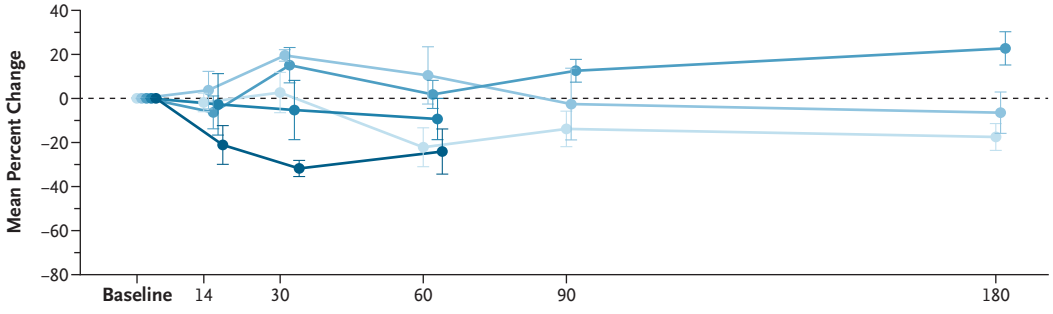
CTX310, 0.1 mg/kg eLBW · CTX310, 0.3 mg/kg eLBW · CTX310, 0.6 mg/kg eLBW · CTX310, 0.7 mg/kg eLBW · CTX310, 0.8 mg/kg eLBW

A Change in Apolipoprotein B Level over Time



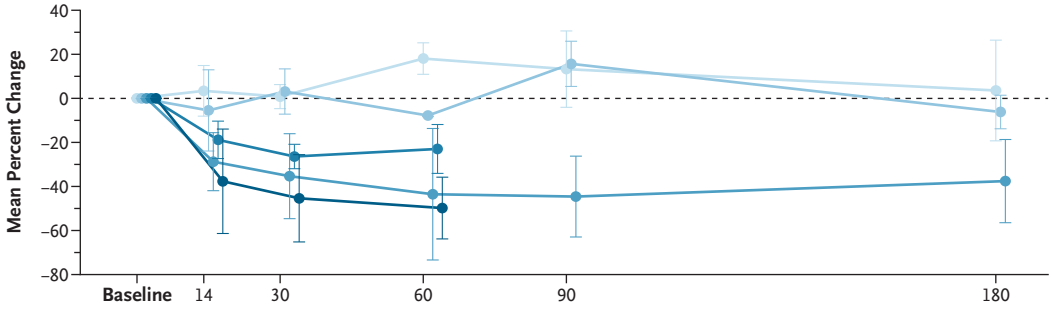
No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	2	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

B Change in HDL Cholesterol Level over Time



No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

C Change in Non-HDL Cholesterol Level over Time



No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

Figure 2 (facing page). Changes in Apolipoprotein B, HDL Cholesterol, and Non-HDL Cholesterol Levels over Time with CTX310.

Panel A shows the mean percent change in apolipoprotein B level from baseline according to visit for each dose level. Panel B shows the mean percent change in high-density lipoprotein (HDL) cholesterol level from baseline according to visit for each dose level. Panel C shows the mean percent change in non-HDL cholesterol level from baseline according to visit for each dose level. I bars indicate standard errors. For each dose group, the line for percent change extends to the final time point at which data were available for all participants.

the assessment of efficacy in specific populations. There was limited participant racial and ethnic diversity of the participant population and limited geographic distribution of sites, and few women were enrolled (Table S3). As compared with potential lifetime exposure to the edited genome, the duration of follow-up in this initial report is short. Ongoing surveillance of participants is essential to assess the long-term safety and lipid-lowering efficacy of CTX310.

In this limited-duration phase 1 trial, one-time in vivo CRISPR-Cas9–mediated editing of the *ANGPTL3* gene was safe.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

A data sharing statement provided by the authors is available with the full text of this article at NEJM.org.

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