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)

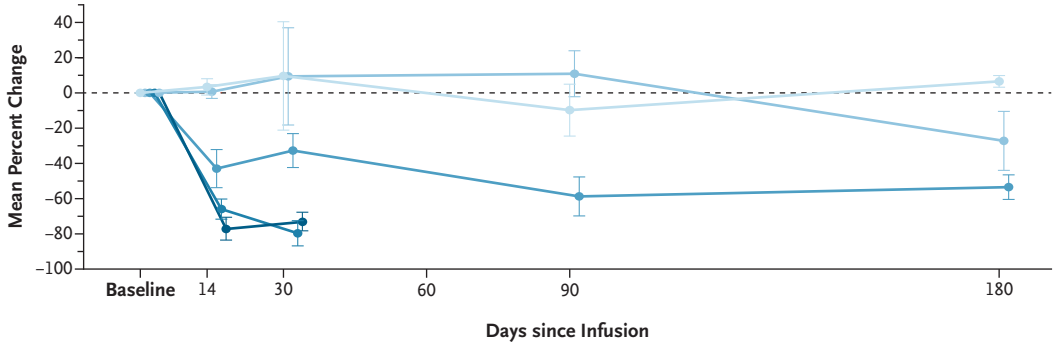
Apolipoprotein B					
Mean baseline level (range) — mg/dl	132.0 (87.0 to 204.0)	139.0 (76.0 to 192.0)	153.7 (109.0 to 211.0)	121.0 (95.0 to 147.0)	116.3 (93.0 to 147.0)
Mean level at follow-up (range) — mg/dl†	144.7 (92.0 to 230.0)	190.5 (180.0 to 201.0)	85.7 (55.0 to 122.0)	95.5 (61.0 to 130.0)	72.0 (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.

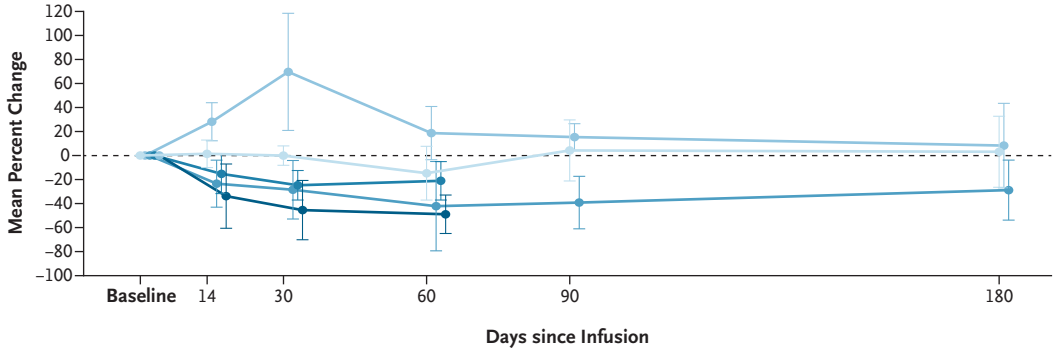
CTX310, 0.1 mg/kg eLBW · CTX310, 0.3 mg/kg eLBW · CTX310, 0.6 mg/kg eLBW · CTX310, 0.7 mg/kg eLBW · CTX310, 0.8 mg/kg eLBW

A Change in ANGPTL3 Level over Time



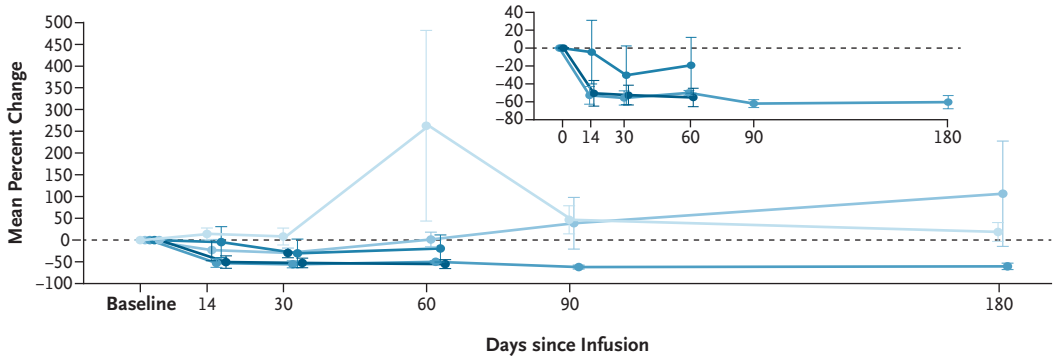
No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.3 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.6 mg/kg eLBW	3	3	3	3	3	
CTX310, 0.7 mg/kg eLBW	2	2	2			
CTX310, 0.8 mg/kg eLBW	4	4	4			

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]

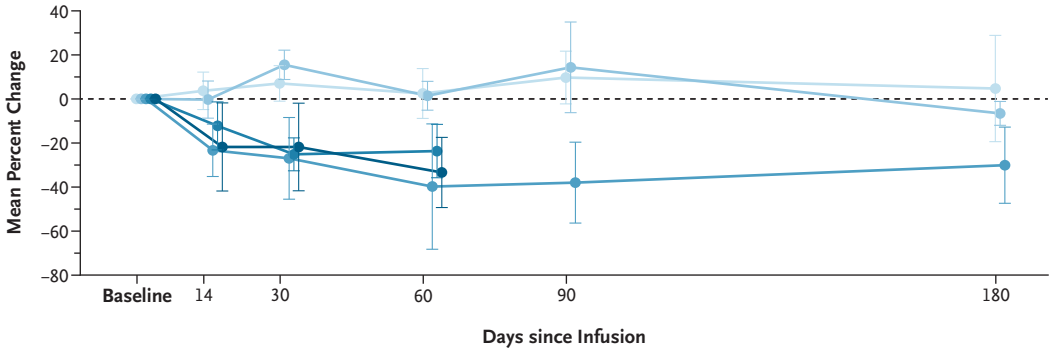
The efficacy of in vivo gene editing and the effect of ANGPTL3 inhibition on atherogenic lipoproteins are probably influenced by factors beyond the administered dose, including hepatic steatosis, inflammation, and preexisting genetic and metabolic profiles.[20,21] This trial population was heterogeneous, 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

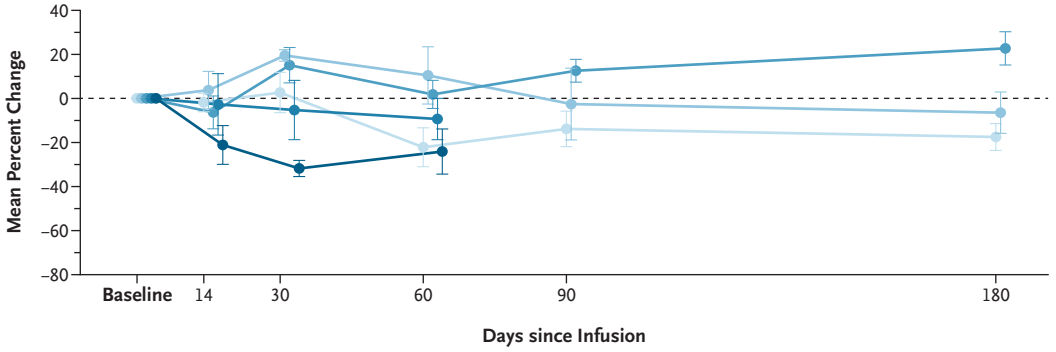
CTX310, 0.1 mg/kg eLBW · CTX310, 0.3 mg/kg eLBW · CTX310, 0.6 mg/kg eLBW · CTX310, 0.7 mg/kg eLBW · CTX310, 0.8 mg/kg eLBW

A Change in Apolipoprotein B Level over Time



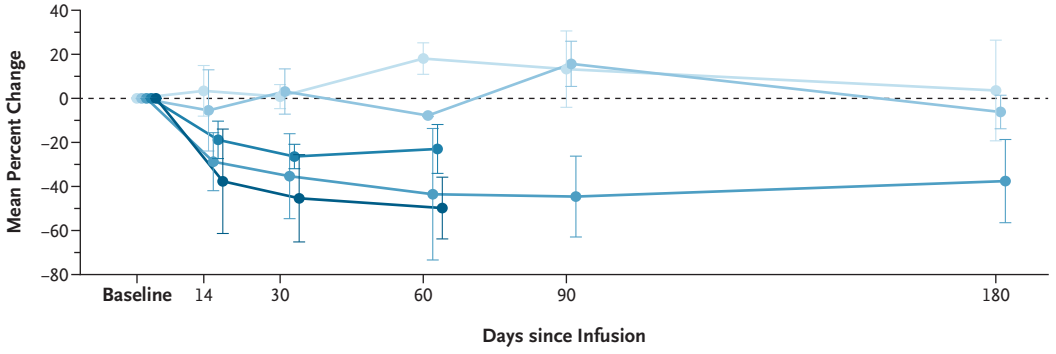
No. of Participants						
CTX310, 0.1 mg/kg eLBW	3	3	3	3	3	3
CTX310, 0.3 mg/kg eLBW	3	3	3	3	2	3
CTX310, 0.6 mg/kg eLBW	3	3	3	2	3	3
CTX310, 0.7 mg/kg eLBW	2	2	2	2		
CTX310, 0.8 mg/kg eLBW	4	4	4	4		

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.

Supported by CRISPR Therapeutics.

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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Supplementary Appendix

Supplement to: Laffin LJ, Nicholls SJ, Scott RS, et al. Phase 1 trial of CRISPR-Cas9 gene editing targeting ANGPTL3. N Engl J Med. DOI: 10.1056/NEJMoa2511778

This appendix has been provided by the authors to give readers additional information about the work.

Table of Contents 1

Study investigators and data monitoring committee members

Study investigators* and sites

Site Name	Location	Principal Investigator
Victorian Heart Hospital, Monash Health	Melbourne, Australia	Stephen Nicholls
New Zealand Clinical Research	Christchurch, New Zealand	Russell Scott
Royal Adelaide Hospital	Adelaide, Australia	Peter Clifton
Aotearoa Clinical Trials	Auckland, New Zealand	John Baker
Richmond Pharmacology	London, United Kingdom	Jorg Taubel
Austin Health	Melbourne, Australia	Elif Ekinci

* The safety review committee was comprised of principal investigators of each site

Independent data monitoring committee members

Member Name	Location
Jeffrey Crippin (DSMB Chair)	St. Louis, MO, USA
Lawrence Leiter	Toronto, Canada
Adam Nelson	Adelaide, Australia
Gheorghe Doros	Boston, MA, USA

Inclusion and exclusion criteria

Inclusion criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

1. Age of ≥18 and ≤75 years at the time of signing the informed consent.

2. Able to provide written informed consent.

3. Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of triglycerides (TG) (>150 mg/dL [1.7 mmol/L]) and/or low-density lipoprotein-cholesterol (LDL-C) (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for subjects with ASCVD) and/or non-high-density lipoprotein-cholesterol (non–HDL-C) (>160 mg/dL [4.1 mmol/L]) and/or apolipoprotein B (ApoB) (>100 mg/dL [2.6 mmol/L]) at screening, despite treatment.

4. Subjects' lipid levels must be refractory to the maximal intensity or maximally tolerated doses of standard of care lines of lipid-lowering therapies or combinations where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid or docosahexaenoic acid), bile acid sequestrants, and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9; alirocumab or evolocumab), for at least 12 weeks prior to screening.

5. Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.

6. Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation.

7. Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation except as required by the protocol.

8. Female subjects must be postmenopausal, defined as:

• At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; or

• Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).

9. All male subjects must agree to the use of an acceptable method of effective contraception and their female partners should also agree to use an effective method of contraception, as defined in the protocol, from consent through 12 months after CTX310 infusion.

10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.

Exclusion Criteria

To be eligible for entry into the study, the subject must not meet any of the exclusion criteria listed below:

1. Subjects with familial chylomicronemia syndrome (FCS) with <5% lipoprotein lipase (LPL) activity, as documented in the medical history. If LPL activity testing is not documented, the subject with FCS will be excluded.

2. Evidence of liver disease, defined as:

a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >2 × ULN, or

b. Prothrombin time (international normalized ratio) >1.5 × ULN, or

c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography.

3. Complete blood count: Neutrophils <1000 cells/μL (1.0×10^9/L); lymphocytes <500 cells/μL (0.5×10^9/L); hemoglobin <11 g/dL (110 g/L) for males, <10 g/dL (100 g/L) for females; or platelet count <100,000/μL (100×10^9/L).

4. Estimated glomerular filtration rate <60 mL/min/1.73 m2, as measured by Modification of Diet in Renal Disease equation.

5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick or urine albumin to creatinine ratio of >300 mg/g.

6. Inadequate diabetes control, with glycosylated hemoglobin >9%.

7. History of alcohol or substance use disorder.

8. History of a significant coagulation disorder.

9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.1 mIU/L or >10 mIU/L).

10. Cardiac left ventricular ejection fraction <50% by echocardiogram.

11. Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm2).

12. Peripheral pulse oximetry saturation of <90%.

13. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic of >90 mmHg, confirmed by a repeat measurement.

14. 12-lead electrocardiogram (ECG) findings demonstrating:

• QTc of >450 ms for males and >470 ms for females at screening.

• Any other ECG finding deemed clinically significant by the investigator.

15. Acute coronary syndrome event within 24 weeks prior to Day 1.

16. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.

17. Acute pancreatitis within 12 weeks prior to Day 1.

18. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for human immunodeficiency virus (HIV) type 1 or type 2, hepatitis B virus (hepatitis B core antibody or hepatitis B surface antigen or NAT), or hepatitis C virus (hepatitis C antibody testing or NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior malignancy within the past 5 years, or current malignancy (except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ and carcinoma in situ of the cervix or breast), or myeloproliferative disorder, or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, have an intact uterus and at least 1 ovary, and are less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
28. Administration of vaccines 30 days before CTX310 infusion.

Biomarker measurement

Fasting blood samples were collected to evaluate lipid parameters (i.e., total cholesterol, triglyceride, HDL cholesterol, direct LDL cholesterol, apolipoprotein B, apolipoprotein C3 and lipoprotein(a)). Samples were analyzed at a central laboratory on an autoanalyzer (Cobas, Roche Diagnostics).

Plasma levels of ANGPTL3, and two lipid components of the lipid nanoparticle were analyzed for pharmacodynamic and pharmacokinetic analyses, respectively. Assays were validated according to the Food and Drug Administration (FDA) and International Council for Harmonisation (ICH) guidance on bioanalytical method validation. ANGPTL3 was measured in plasma samples utilizing an enzyme-linked immunosorbent assay (R&D Systems). Two lipid nanoparticle lipid components (ionizable lipid and polyethylene glycol (PEG)-lipid) were quantified via liquid chromatography tandem mass spectrometry (LC-MS/MS) following extraction.

The central lab test results of lipid levels were used for the analyses. The lipid levels are directly available as lab test results except for remnant cholesterol, which was calculated as follows: Remnant Cholesterol = Total Cholesterol – LDL-C – HDL-C.

Estimated lean body weight calculation

The lean body weight was estimated (eLBW) using the following formula*:

Female: eLBW (kg) = (9270 * [total body weight in kg]) / (8780 + (244 * [BMI in kg/m^2]))

Male: eLBW(kg) = (9270 *[total body weight in kg]) / (6680 + (216 * [BMI in kg/m^2])),

where BMI (kg/m^2) = (total body weight in kg) / (height in m)^2.

* Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet. 2005;44:1051–1065.

Study objectives and end points

Primary Objectives	Primary End Points
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of dose-limiting toxicities and frequency of adverse events
Secondary Objectives	**Secondary End Points**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To further characterize the safety of CTX310	• Frequency and severity of adverse events (AEs), including treatment-emergent adverse events (TEAEs) and adverse events of special interest (AESIs), clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
• To assess the PK of CTX310	• Plasma levels of lipid nanoparticle (ionizable and PEG lipid) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline
Exploratory Objectives	**Exploratory End Points**
• To identify changes associated with CTX310 that may indicate or predict clinical response, immunogenicity, safety, or PD activity	• Percentage change in free fatty acid levels over time compared to baseline. • Change in fatty liver disease. • Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to lipid nanoparticles and Cas9, if required). • For Phase 1b only: Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG • Percentage change in remnant cholesterol concentration over time compared to baseline

ADA denotes anti-drug antibody, ANGPTL3 angiopoietin-like 3, ApoB apolipoprotein B, Cas9 CRISPR-associated protein 9, FFA free fatty acid, HDL-C high-density lipoprotein cholesterol, LDL-C low- density lipoprotein cholesterol, Lp(a) lipoprotein(a), PD pharmacodynamic(s), PEG polyethylene glycol, PK pharmacokinetics and TG triglycerides.

CTCAE grading scale and definitions of dose-limiting toxicities

Common Terminology Criteria for Adverse Events (CTCAE) Version 5.0 Definitions:

Grade refers to the severity of the AE. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on this general guideline:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL*.
Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL**.
Grade 4 Life-threatening consequences; urgent intervention indicated.
Grade 5 Death related to AE.

Activities of Daily Living (ADL) *Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc. **Self-care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden

Definitions of dose-limiting toxicities

The dose-limiting toxicities definitions used in this study are informed by nonclinical studies of CTX310 and published reporting of clinical experience with a lipid nanoparticle-encapsulated, CRISPR-Cas-9–based genome editing therapy. Adverse events (AE) that have no plausible causal relationship with CTX310 will not be considered dose-limiting toxicities. A dose-limiting toxicity will be graded and documented according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a dose-limiting toxicity will include the following:

1. Any CTCAE grade ≥3 elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that persist for >14 days and is assessed by the investigator as related to investigational product.

2. Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) that is assessed by the investigator as related to investigational product.

3. Any other CTCAE grade 3 laboratory abnormality that persists ≥7 days and is assessed by the investigator as related to investigational product.

4. Any other CTCAE grade 4 laboratory abnormality that is assessed by the investigator as related to investigational product.

5. Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product.

Narrative description of one participant death

One participant death occurred in a patient that was in the 0.1 mg/kg cohort. The participant was a 51-year-old man with a clinical diagnosis of homozygous familial hypercholesterolemia diagnosed at age 32. Genetic testing obtained during the trial demonstrated a pathogenic deletion of *LDLR* exon 2-6, consistent with heterozygous familial hypercholesterolemia. He also had a history of obstructive sleep apnea, hypertension, and coronary artery disease requiring multiple coronary revascularization procedures, with four coronary stents placed between the ages 32 and 41 years of age. Additionally, he underwent a coronary artery bypass grafting operation at age 48. At the time of enrollment, the participant was taking lipid lowering therapy including rosuvastatin 10 mg daily, ezetimibe 40 mg daily, evinacumab 15 mg/kg IV monthly, and alirocumab 150 mg SC every 2 weeks. He was enrolled in dose level 1 (0.1 mg/kg estimated lean body weight) and received a dose of 9.5 mg of total RNA. The participant tolerated the infusion well without any drug-related adverse events. On day 13 following infusion he experienced an unrelated adverse event of diarrhea and abdominal pain due to suspected food poisoning or gastroenteritis. The remaining safety monitoring period through day 30 post-infusion was uneventful with no further lab abnormalities. No further adverse events were reported through his month 6 post-infusion visit. On day 179 post-infusion, the participant collapsed suddenly while walking down the street while unaccompanied. He was found by bystanders who attempted cardiopulmonary resuscitation. Emergency medical services were called to the scene, but they were unable to resuscitate the participant. No autopsy was performed. The participant's death was deemed unrelated to investigational product by the primary investigator.

Pre-clinical assessment of off-target effects of CTX310 and gRNA sequence

Assessment of off-target effects

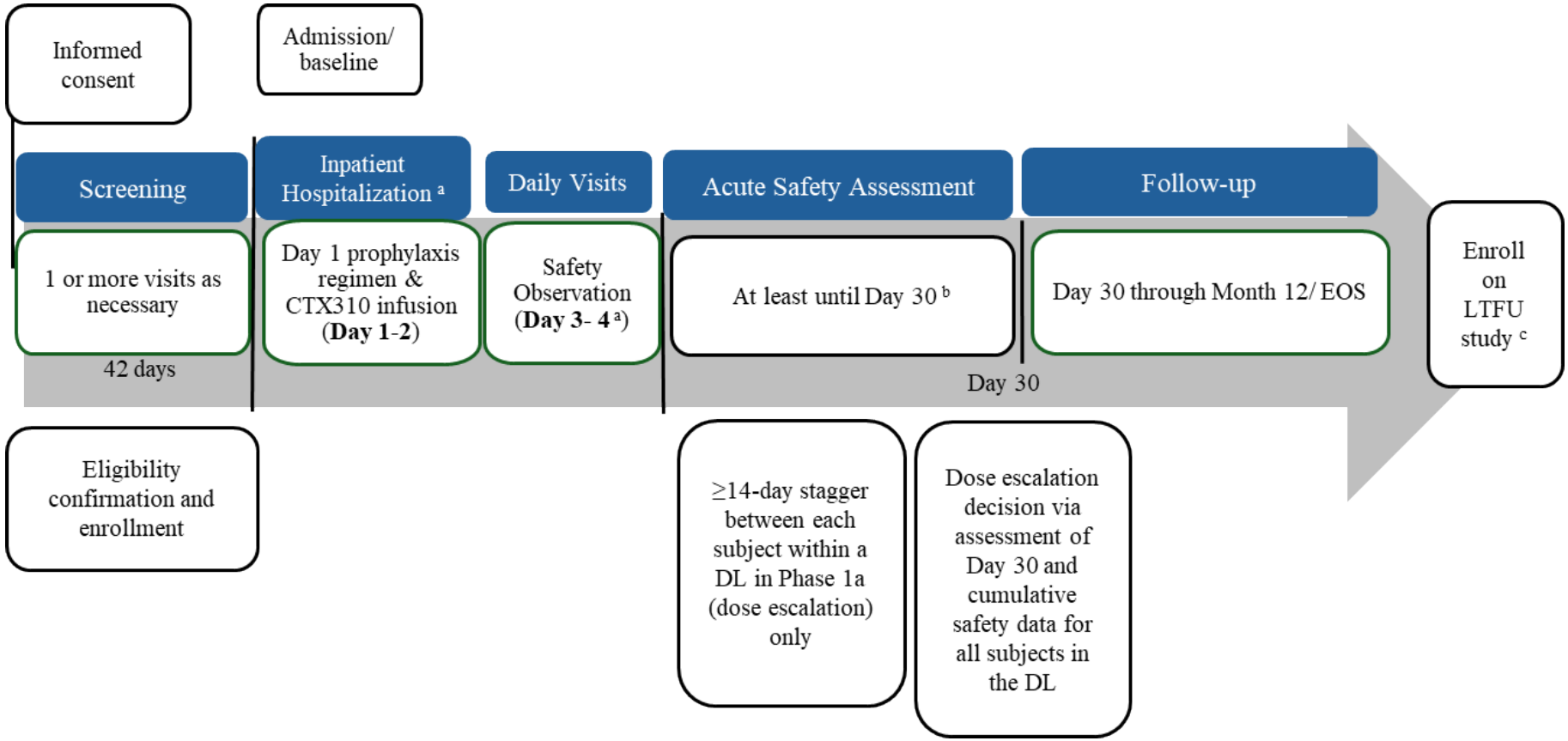
Through the development of exagamglogene autotemcel (CASGEVY), the first FDA approved CRISPR-based gene editing therapy for the treatment of sickle cell disease and transfusion-dependent beta thalassemia, extensive computational and experimental methods were developed to comprehensively characterize the risk of off-target edits (Frangoul NEJM 2021, Frangoul NEJM 2024, Locitelli NEJM 2024, Yen NEJM 2024). This process was adapted to develop CTX310, an in vivo gene editor targeting ANGPTL3, and was used to assess the potential of CTX310 to introduce unintended off-target edits the across the human genome.

Off-target editing characterization involves 2 stages. In the first stage (site nomination), 2 orthogonal approaches (computational search and Digenome-seq) were used to nominate a broad range of candidate genome-wide off-target sites. In the second stage (editing confirmation), the nominated off-target sites were followed up with hybrid capture deep sequencing to assess for evidence of editing by comparing indel rates in CTX310-treated and donor-matched untreated control samples. The off-target confirmation experiments were performed on primary human hepatocytes (PHHs) from four individual donors (two male and two female). After genome-wide site nomination followed by deep-sequencing confirmation, no off-target editing site was detected at the 0.2% allele frequency threshold for CTX310.

gRNA sequence

5’-u*a*a* GAC CAU GUC CCA ACU GAG UUU UAG Agc uag aaa uag cAA GUU AAA AUA AGG CUA GUC CGU UAU Caa cuu gaa aaa gug gca ccg agu cgg ugc u*u*u*u-3’ (SEQ ID NO: 13) wherein, u = 2’OMe-rU; a = 2’OMe-rA; c = 2’OMe-rC; g = 2’OMe-rG; * = Phosphorothioate. The underlined sequence corresponds to the spacer.

Figure S1. Study design

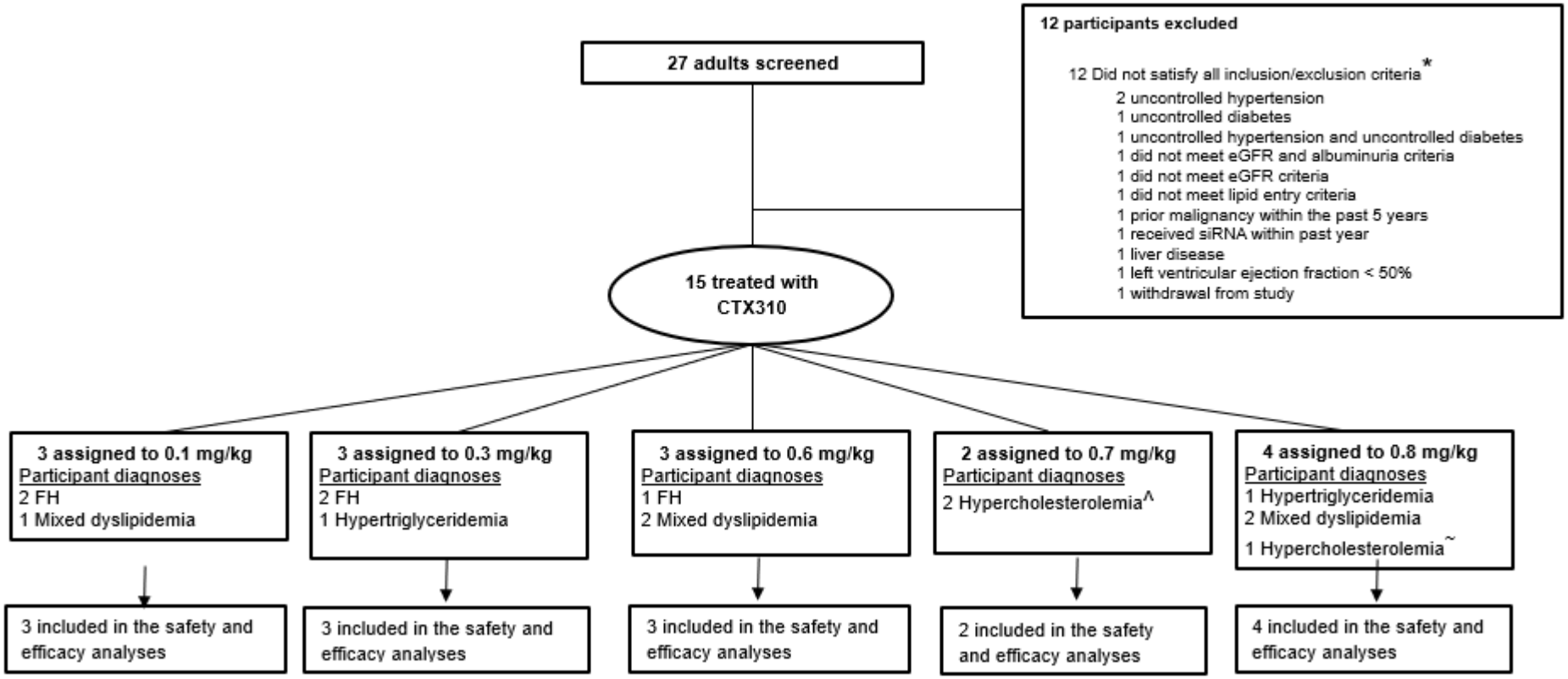


[a] Inpatient hospitalization for CTX310 infusion on Day 1. Hospital discharge on Day 2 after the completion of safety evaluation and laboratory tests and a minimum of 24 hours after the completion of the CTX310 infusion. Daily safety visits on Day 3 and 4. Inpatient hospitalization may be extended or following hospital discharge the subject may be readmitted or additional daily safety visits beyond Day 4 may be required at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

[b] For each dose level (DL) during Phase 1a dose escalation, there will be a safety monitoring period of ≥14 days between the treatment of each subject and any subsequent subjects within the DL, or until the subject is clinically stable and all laboratory values (including liver function tests) have returned to <2 × baseline or to normal levels, whichever is later. No stagger is required in Phase 1b.

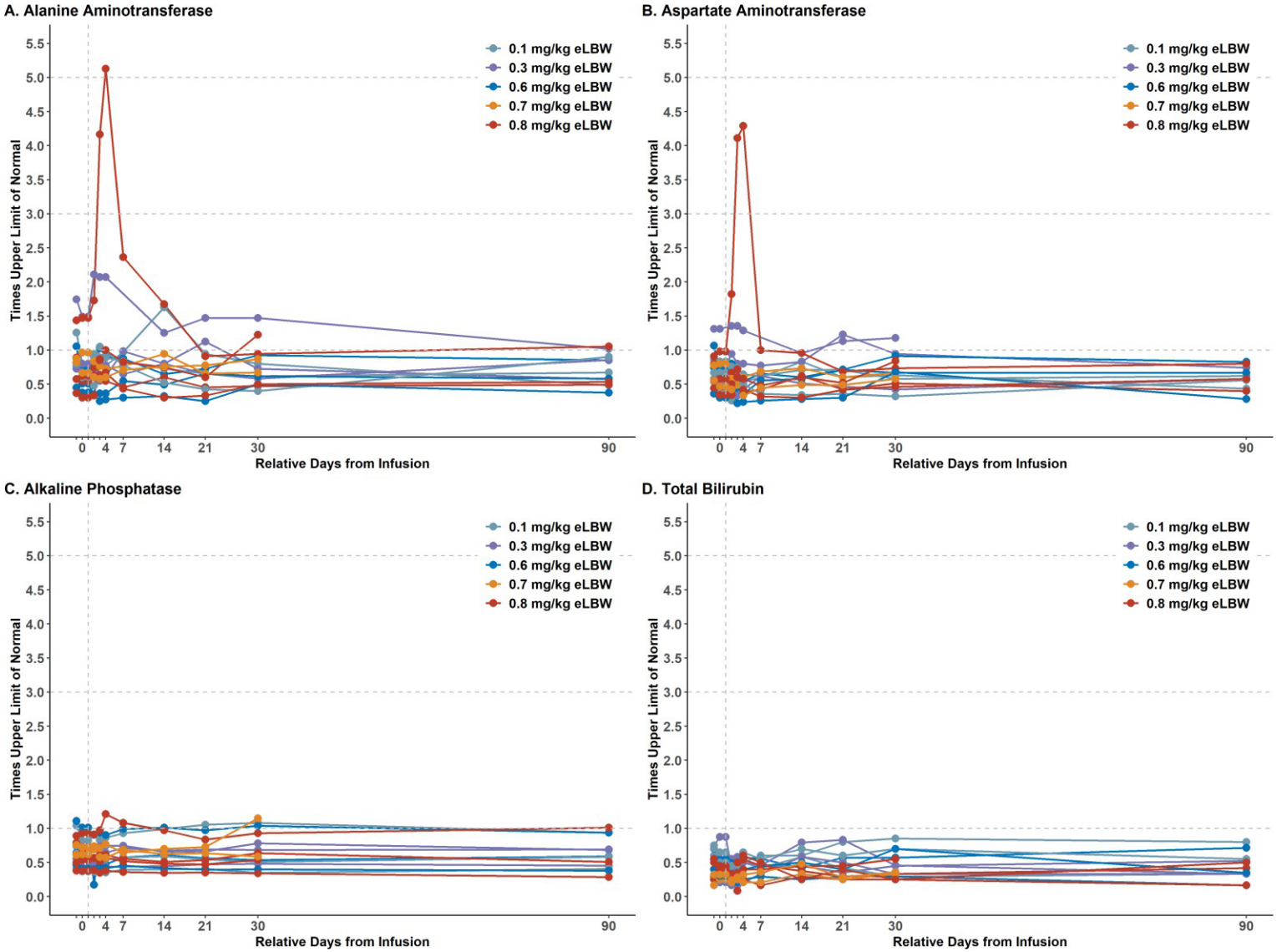
[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for roll over into a separate long-term follow-up (LTFU) study for up to 15 years post-infusion.

Figure S2. Flow of participants through the trial



* Some participants may not satisfy more than one inclusion criteria or had several exclusion criteria.
^ One participant was enrolled with a diagnosis of mixed dyslipidemia; however, laboratory values were consistent with hypercholesterolemia alone.
~ One participant was enrolled with a clinical diagnosis of familial hypercholesterolemia, but diagnosis was not confirmed with genetic testing.
The safety analysis set includes the randomized participants who received the CTX310 infusion. Analyses of the safety assessments are based on the safety analysis set. Efficacy analyses were performed based on the full analysis set, which includes the participants who received CTX310 infusion and have at least 1 post-baseline lipid assessment. Total dose was calculated based on estimated lean body weight.
FH denotes familial hypercholesterolemia and eGFR – estimated glomerular filtration rate

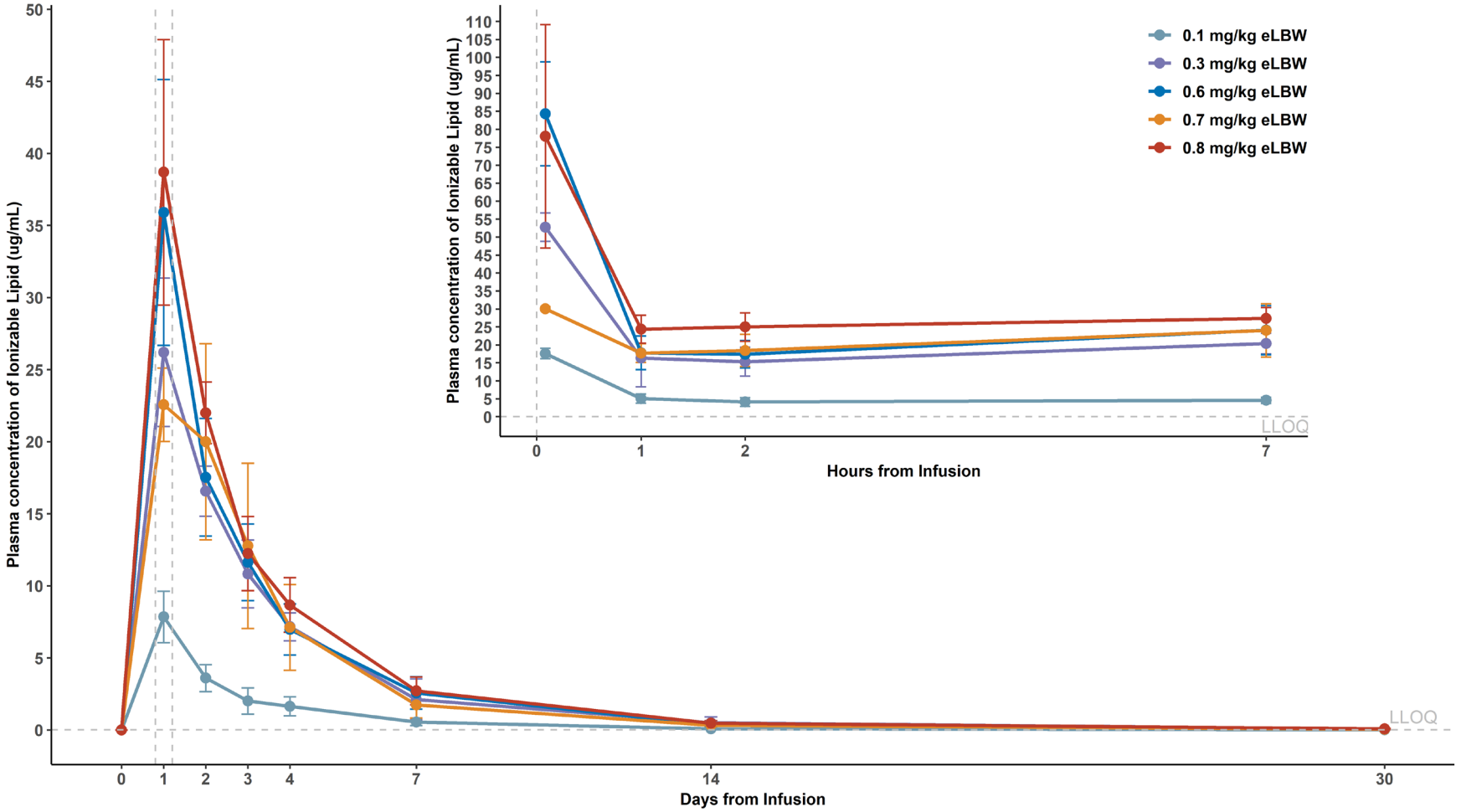
Figure S3. Liver function test changes over time for all participants



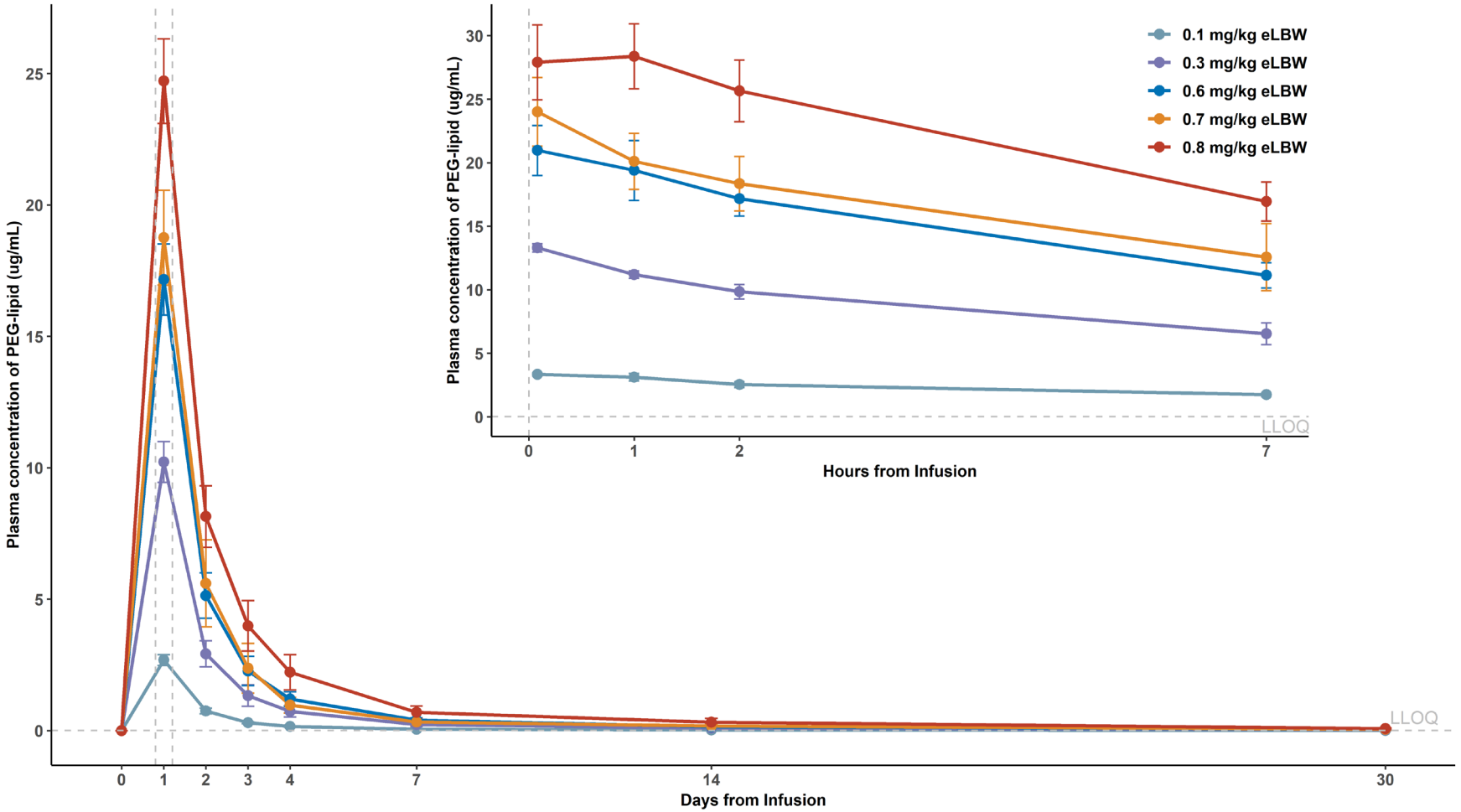
Measurements from the day prior to treatment through day 90 are shown for all participants. A single trial participant experienced a Common Terminology Criteria for Adverse Events (CTCAE) Grade 2 elevation of aminotransferases with the 0.8 mg/kg estimated lean body weight dose. This elevation was considered Grade 2 because the participant had a baseline alanine aminotransferase level 1.5 times the upper limit of normal. The dashed lines represent 1.0x, 3.0x and 5.0x the upper limit of normal for the respective biomarkers. eLBW denotes estimated lean body weight.

Figure S4. Plasma levels of lipid nanoparticle components

A. Ionizable lipid



B. Polyethylene glycol (PEG) lipid



Panel A displays the amount of ionizable lipid measured by visit for each dose level. Panel B displays the amount of polyethylene glycol (PEG) lipid measured by visit for each dose level. Error bars represent the standard error of the means. Values are shown until 30 days following treatment. LLOQ denotes lower limit of quantification. The LLOQ was determined during validation in accordance with Food and Drug Administration and International Council on Harmonisation guidance on bioanalytical method validation

Table S1. All treatment emergent adverse events by system organ class and preferred term

	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 (N=15)
Investigations	**1**	**2**	**2**	**0**	**2**	**7**
C-reactive protein increased	0	0	1	0	1	2
White blood cell count increased	0	2	0	0	0	2
Alanine aminotransferase increased	1	0	0	0	0	1
Blood creatinine increased	0	0	0	0	1	1
Blood glucose fluctuation	0	0	1	0	0	1
Hepatic enzyme increased	0	0	0	0	1	1
Musculoskeletal and connective tissue disorders	**2**	**1**	**1**	**0**	**2**	**6**
Back pain	1	0	0	0	0	1
Flank pain	0	0	0	0	1	1
Intervertebral disc protrusion	0	1	0	0	0	1
Muscle twitching	0	0	0	0	1	1
Musculoskeletal pain	0	0	1	0	0	1
Myalgia	1	0	0	0	0	1
Neck pain	1	0	0	0	0	1
Injury, poisoning, and procedural complications	**1**	**0**	**2**	**0**	**1**	**4**
Infusion related reaction	0	0	2	0	1	3
Concussion	1	0	0	0	0	1
Head injury	1	0	0	0	0	1
Tooth fracture	1	0	0	0	0	1
Nervous system disorders	**1**	**1**	**2**	**0**	**0**	**4**
Headache	0	1	2	0	0	3
Dizziness	1	0	0	0	0	1
Gastrointestinal disorders	**1**	**2**	**0**	**1**	**0**	**4**
Diarrhea	0	1	0	1	0	2

Dry mouth	0	1	0	0	0	1
Food poisoning	1	0	0	0	0	1
Nausea	0	1	0	0	0	1
Toothache	0	1	0	0	0	1
Vomiting	0	1	0	0	0	1
General disorders and administration site conditions	**2**	**0**	**0**	**0**	**1**	**3**
Death	1	0	0	0	0	1
Fatigue	1	0	0	0	1	2
Blood lymphatic system disorders	**1**	**0**	**1**	**0**	**0**	**2**
Leukocytosis	0	0	1	0	0	1
Neutrophilia	1	0	0	0	0	1
Eye disorders	**1**	**0**	**1**	**0**	**0**	**2**
Vision blurred	1	0	0	0	0	1
Vision impairment	0	0	1	0	0	1
Infections and infestations	**0**	**0**	**0**	**1**	**1**	**2**
Gastroenteritis	0	0	0	0	1	1
Localized infection	0	0	0	1	0	1
Cardiac disorders	**0**	**1**	**0**	**0**	**0**	**1**
Palpitations	0	1	0	0	0	1
Ear and labyrinth disorders	**0**	**0**	**0**	**0**	**1**	**1**
Cerumen impaction	0	0	0	0	1	1
Metabolism and nutrition disorders	**1**	**0**	**0**	**0**	**0**	**1**
Hyperglycemia	1	0	0	0	0	1
Psychiatric disorders	**0**	**1**	**0**	**0**	**0**	**1**
Insomnia	0	1	0	0	0	1
Renal and urinary disorders	**0**	**0**	**0**	**0**	**1**	**1**
Acute kidney injury	0	0	0	0	1	1

Skin and subcutaneous tissue disorders	**0**	**1**	**0**	**0**	**0**	**1**
Rash	0	1	0	0	0	1
Vascular disorders	**0**	**0**	**0**	**0**	**1**	**1**
Hypertension	0	0	0	0	1	1

Adverse events were coded using the preferred term in the Medical Dictionary for Regulatory Activities (MedDRA), version 28.0. The table includes adverse events with an onset date on or after CTX310 treatment.

Table S2. Changes in remnant cholesterol and apolipoprotein CIII

	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 CTX310 RNA (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)
Remnant cholesterol*					
Mean baseline (range), mg/dL	26.2 (20.1 to 30.2)	84.8 (36.0 to 168.2)	53.2 (49.5 to 59.9)	28.2 (15.1 to 41.4)	45.3 (15.9 to 102.1)
Mean at follow-up† (range), mg/dL	41.4 (26.3 to 54.9)	30.2 (17.0 to 43.3)	21.9 (16.2 to 29.4)	18.9 (12.8 to 25.1)	21.0 (8.9 to 30.9)
Mean absolute change (range), mg/dL	15.2 (6.2 to 26.7)	-13.0 (-18.9 to -7.0)	-31.3 (-39.8 to -20.1)	-9.3 (-16.2 to -2.3)	-24.4 (-71.2 to -3.9)
Mean percent change (range), %	55.9 (30.8 to 94.5)	-33.3 (-52.7 to -13.8)	-58.3 (-67.7 to -40.6)	-27.3 (-39.3 to -15.4)	-42.2 (-69.7 to -16.1)
Apolipoprotein CIII					
Mean baseline (range), mg/dL	9.2 (5.6 to 12.4)	15.1 (9.8 to 19.9)	15.0 (10.1 to 21.4)	7.8 (4.6 to 11.0)	17.6 (11.1 to 30.1)
Mean at follow-up† (range), mg/dL	12.9 (5.2 to 20.1)	12.5 (8.7 to 16.3)	6.8 (4.4 to 8.5)	4.7 (2.2 to 7.1)	6.0 (2.2 to 7.9)
Mean absolute change (range), mg/dL	3.7 (-0.4 to 7.7)	-0.2 (-1.1 to 0.8)	-8.1 (-13.8 to -4.9)	-3.2 (-3.9 to -2.4)	-11.6 (-22.2 to -3.8)
Mean percent change (range), %	31.7 (-7.1 to 62.1)	-3.0 (-11.2 to 5.2)	-52.5 (-64.5 to -36.6)	-43.8 (-52.2 to -35.5)	-61.7 (-85.1 to -34.2)

*Remnant cholesterol was calculated based on the following formula: Remnant cholesterol = (Total cholesterol) – (Low density lipoprotein cholesterol) – (High density lipoprotein cholesterol)

†Follow-up measurements are 90 days following 0.1, 0.3, 0.6 mg/kg CTX310 doses and 60 days following 0.7 and 0.8 mg/kg CTX310 doses. To convert the values for remnant cholesterol to millimoles per liter, multiply by 0.02586.

Table S3. Representativeness of trial population

Disease Under Investigation	**Persistent atherogenic dyslipidemia among patients with a diagnosis of familial or non-familial hypercholesterolemia, hypertriglyceridemia, or mixed dyslipidemia**
Special Considerations related to:	
Sex and gender	The trial enrolled both men and women, with no sex-based restrictions in eligibility. Among the United States population enrolled in the All of Us research program, men had a higher prevalence of familial hypercholesterolemia pathogenic variants than women (1 in 485 vs. 1 in 602, P-value = 0.03) (Osei). Among the US population examined in the National Health and Nutrition Examination Survey, United States adults with triglycerides ≥500 mg/dL, 24.2% were women and 75.8% were men (Gurevitz).
Race and Ethnicity	There were no restrictions to enrollment based on race and ethnicity, however the trial ultimately treated participants primarily of white race in part due to geographic location of the sites.
Age	Adults 18 to 75 years of age were eligible, encompassing a wide spectrum of ages affected by severe dyslipidemia. The age distribution of enrolled participants was broadly consistent with epidemiologic data for familial hypercholesterolemia and severe hypertriglyceridemia, with intentional inclusion of both younger adults with early-onset disease and older adults with longstanding refractory dyslipidemia.
Overall representativeness of this trial	The enrolled trial population was broadly representative of adults with severe dyslipidemia who are refractory to maximally tolerated lipid-lowering therapy. The trial had strict eligibility criteria, is a first-in-human trial, and only recruited participants at 6 total sites. As with other early phase gene-editing studies, participation from certain racial and ethnic groups was limited, and these phase 1a findings may not fully generalize to populations without access to specialized lipid clinics.

Data cited from:

Osei J, Razavi AC, Quyyumi AA, et al. Sex and racial differences in prevalence and clinical characteristics of people living with LDLR and PCSK9 familial hypercholesterolemia genetic variants: Data from the All of Us Research program. Am J Prev Cardiol. 2025;22:101024.

Gurevitz C, Chen L, Muntner P, Rosenson RS. Hypertriglyceridemia and Multiorgan Disease Among U.S. Adults. JACC Adv. 2024;3(5):100932.

Martin SS, Aday AW, Allen NB, et al. 2025 Heart Disease and Stroke Statistics: A Report of US and Global Data From the American Heart Association. Circulation. 2025;151(8):e41-e660.

Protocol

Protocol for: Laffin LJ, Nicholls SJ, Scott RS, et al. Phase 1 trial of CRISPR-Cas9 gene editing targeting ANGPTL3. N Engl J Med. DOI: 10.1056/NEJMoa2511778

This trial protocol has been provided by the authors to give readers additional information about the work.

This supplement contains the following items:

1. Original protocol, two versions of the final protocol (Australia / New Zealand Version and United Kingdom version) and a summary of changes.

2. Statistical analysis plan. There is only a single finalized version of the statistical analysis plan.

CLINICAL STUDY PROTOCOL

CRSP-CVD-400

A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias

Study Drug: CTX310
Study Phase: 1
Date of Original Protocol: 27 January 2023

Date of Protocol Amendment: 05 May 2023, Amendment 1
Version: 2.0

Sponsor Emergency Contact	[redacted]	[redacted]

CRISPR Therapeutics AG
Baarerstrasse 14
CH 6300 Zug, Switzerland

SIGNATURE PAGES

PROTOCOL APPROVAL SIGNATURE PAGE

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	05 May 2023
Version	2.0
Amendment	1

Reviewed and approved by:

[redacted]

[redacted]

Date

PROTOCOL ACCEPTANCE FORM

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	05 May 2023
Version	2.0
Amendment	1

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice, and all applicable regulatory requirements.

Investigator's Signature _____ Date _____

Name (printed) _____

AMENDMENT DETAILS

Summary of Changes in the Current Amendment

Protocol CRSP-CVD-400 Version 1.0, dated 27 January 2023, was internally approved per the sponsor's standard operating procedure. However, Version 1.0 was not submitted to a health authority, Institutional Review Board, or Ethics Committee and was revised to Version 2.0, dated 05 May 2023, to reflect changes to the study conduct based on the sponsor's decision. The protocol history is documented in Section 14.4.2.

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1. PROTOCOL SUMMARY

1.1. Protocol Synopsis

Sponsor: CRISPR Therapeutics AG	**Protocol Number**: CRSP-CVD-400
Name of Investigational Product: CTX310	**Phase of Development**: 1
Protocol Title: A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR-Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias.	
Number of Subjects (Participants from heretofore): Approximately 24	
Investigators: Multicenter	**Study Type**: Interventional
Investigational Product Description CTX310 is a lipid nanoparticle (LNP) formulation of CRISPR-Cas9 (clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9) components for in vivo editing of the target gene angiopoietin-like 3 (*ANGPTL3*). The investigational drug product consists of a capped and polyadenylated spacer Cas9 mRNA containing N1-methylpseudouridine and a 100 nucleotide–long single-guide RNA targeting the gene of interest. CTX310 is designed to utilize CRISPR-Cas9 to disrupt exon 1 of human *ANGPTL3* in the liver, leading to a decrease of ANGPTL3 protein levels.	
Mode of Administration: Participants will receive a single intravenous (IV) infusion.	
Study Population The study population will consist of participants 18 to 70 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride (TG) and/or non–high-density lipoprotein cholesterol (HDL-C), including low-density lipoprotein (LDL), very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) (Lp(a)), and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTD) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population).	

This Phase 1 study will include participants with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:

- Multifactorial chylomicronemia syndrome
- Homozygous familial hypercholesterolemia.
- Heterozygous familial hypercholesterolemia
- Other HTG/hypercholesterolemia syndromes of undetermined etiologies.

The majority of participants enrolled are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia and HTG. Participants with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk. Participants will be asked to continue to take their baseline lipid-lowering medications in the same doses through the study period until a significant beneficial effect (i.e., achievement of target lipid goals) of CTX310 is observed.

Duration of Participation: All participants will be monitored for safety, tolerability, pharmacokinetics (PK), and pharmacodynamic (PD) effects for 12 months post-infusion in the study. All participants will be asked to participate in a separate long-term follow-up study following completion or withdrawal/discontinuation.

Objectives and Endpoints

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of AEs, including TEAEs, AESIs, DLTs; clinically significant laboratory abnormalities; and clinically significant abnormal vital signs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non-HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline

AE: adverse event; AESI: adverse event of special interest; [redacted] ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; DLT: dose-limiting toxicity; HDL-C: high-density lipoprotein cholesterol; low-density lipoprotein cholesterol; LNP: lipid nanoparticles; Lp(a): lipoprotein(a); PD: pharmacodynamic; PK: pharmacokinetic; TEAE: treatment-emergent adverse event; TG: triglycerides.

Study Design: This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll up to 24 participants 18 to 70 years of age with dyslipidemias and increased levels of TG (>300 mg/dL) and/or LDL-C (>100 mg/dL; >70 mg/dL for ASCVD) and/or non–HDL-C (>160 mg/dL) and/or ApoB (>100 mg/dL) that are refractory to indicated and available treatments

Three to 6 participants will be enrolled in each of the dose levels: 0.1, 0.3, 0.6, and 0.8 mg/kg estimated lean body weight (eLBW) of total RNA in the LNP formulation. Each participant will receive a single IV dose of CTX310 and will be hospitalized for a minimum of 24 hours after CTX310 infusion (or longer if required by local regulation or site practice) and will be closely monitored post-infusion for adverse events (AEs) defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All participants will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Details of the toxicity management guidelines are provided in the protocol.

After CTX310 infusion, participants will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on *ANGPTL3* expression and lipid profile. After completion of this study, all participants will be asked to participate in a separate long-term follow-up study for up to 15 years post-infusion.

At each dose level, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next cohort. Once dose escalation is completed and a recommended dose has been determined, at least 3 additional participants will be enrolled at the same dose level to confirm safety and PD effect (confirmatory cohort).

Each participant will undergo the following stages:

1. Screening: up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All participants will be monitored for safety, tolerability, PK, and PD effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: Roll over to a separate long-term follow-up study for up to 15 years post-infusion.

Study Oversight

Safety Review Committee

An SRC consisting of investigators and sponsor representatives will review all available safety data when the DLT observation period ends for the last participant enrolled in each cohort and will be responsible for making decisions regarding dose escalation or de-escalation. Throughout dose escalation, for cases in which a dose had been cleared in a cohort and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to

enroll an additional number of participants for a total of up to 6 participants at the current dose level to gather additional safety data. The SRC will continue to meet regularly during the dose escalation phase to discuss toxicity management algorithms and to review individual participant cases. Following discussion with the SRC, the sponsor may consult with the independent Data Safety Monitoring Board (DSMB) regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema. Based on ongoing assessment of benefit and risk, the SRC may stop dose escalation before a MTD is determined.

Data Safety Monitoring Board

An independent DSMB consisting of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise will be formed at the start of the study, and roles and responsibilities will be described in the DSMB charter. Throughout the study the DSMB will review safety and efficacy data from dose escalation and endorse the recommended Phase 2 dose (RP2D).

The sponsor or designee will be responsible for alerting the DSMB regarding any suspected, unexpected, serious adverse reaction related to CTX310.

Proposed Starting Dose and Dose Escalation

The following doses in the following table are proposed for evaluation in the 4 planned dose escalation levels in the study, with a minimum of 3 and a maximum of 6 evaluable participants per dose level (DL). The first-in-human starting dose is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the non-human primate (NHP) Good Laboratory Practice (GLP) safety and toxicology study. The initial starting dose of 0.1 mg/kg eLBW of CTX310 refers to the total RNA dose that is based on an NOAEL of 1 mg/kg in the GLP toxicity study. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg LBW. The emerging clinical data on systemically infused LNP-associated therapeutics demonstrate a relatively safe profile. A 3-fold safety factor is proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies. With an anticipated 3- to 2-fold increment in dose levels, dose escalation is expected to proceed from 0.1 to 0.8 mg/kg eLBW. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data.

Dose Escalation of CTX310

Dose Level	Planned Dose (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6 [2]
4	0.8 [3]

DL: Dose Level; eLBW: estimated lean body weight

[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW. See Appendix 14.1 for eLBW calculation.

[2] Following review of clinical data by the sponsor and Safety Review Committee at DL3, a de-escalation to a dose of 0.5 mg/kg eLBW may be explored.

[3] Following review of clinical data by the sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.

Dosing Within a Dose Level Cohort

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 participants will be treated at each dose level depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in liver function tests (LFTs) after dosing with CTX310 resolved within 14 days, the dosing between each participant within a cohort will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs) have returned to <2 × baseline or to normal levels, whichever is later. If the safety evaluation of a participant is acceptable, the next participant in the cohort may be dosed.

Dose escalation may proceed when all participants in the preceding dose cohort have completed dosing, the last participant has completed ≥30-day safety evaluation, and the cumulative safety data of all treated participants at that dose level demonstrate an acceptable safety profile, as determined by the SRC.

Dose-limiting Toxicity Assessment

Participants must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable participant (i.e., a participant that has been administered CTX310 and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable participants are required per cohort.

An adequate interval will be applied between current lipid-lowering treatments a participant is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities.

Note: Any participant who experiences a DLT will be considered evaluable. Data for all participants who receive CTX310 will be part of the safety analysis set.

Dose escalation will be performed according to the following rules:

- If 0 of 3 participants experience a DLT, escalate to the next dose level.
- If 1 of 3 participants experiences a DLT, expand the current dose level to 6 participants.
  - If 1 of 6 participants experiences a DLT, escalate to the next dose level.
  - If ≥2 of 6 participants experience a DLT in DLs 2, 3, or 4, de-escalate to previous dose level, or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- If ≥2 of 3 participants experience a DLT in DLs 2, 3, or 4 or any optional de-escalation dose level as specified in the table above, de-escalate to previous dose level or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- No dose escalation beyond highest dose planned or listed for the study (see the dose level table above).

The sponsor will declare the RP2D at or below the MTD, or alternatively an optimum biological dose (OBD), based on the analysis of clinical data. At least 3 additional participants

will be administered CTX310 at this dose level (MTD or OBD) before an RP2D is confirmed. A dose expansion cohort may be added to the protocol in a future amendment.

Toxicities will be graded and documented per criteria described in the protocol.

All cumulative AEs occurring outside the DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the DSMB.

Dose-limiting Toxicities: Rationale and Criteria

The DLT definitions used in this study are informed by nonclinical studies of CTX310 and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas-9–based genome editing therapy. AEs that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

- Any CTCAE grade ≥3 AE that is related to study drug.
- Any CTCAE grade 3 laboratory abnormality that persists ≥7 days and is related to study drug.
- Any CTCAE grade 4 laboratory abnormality that is related to study drug.

Study Eligibility

Inclusion Criteria

To be considered eligible to participate in this study, a participant must meet all the inclusion criteria listed below:

1. Age of ≥18 and ≤70 years at the time of signing the informed consent.
2. Able to provide written informed consent.
3. Participants diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>300 mg/dL) and/or LDL-C (>100 mg/dL; >70 mg/dL for participants with ASCVD) and/or non–HDL-C (>160 mg/dL) and/or ApoB (>100 mg/dL) at screening.
4. Participants must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, icosapent ethyl, and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening.
5. Participants with homozygous familial hypercholesterolemia receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Participants on available standard of care lines of treatment, including statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors,

must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation.

7. Participants on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation.
8. Female participants must be postmenopausal, defined as:
   - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
   - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male participants must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in the protocol, from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.
11. Willing to participate in a long-term follow-up study for up to 15 years after completion of this study.

Exclusion Criteria

To be eligible for entry into the study, the participant must not meet any of the exclusion criteria listed below:

1. Participants with familial chylomicronemia syndrome (FCS) as documented in the medical history.
2. Evidence of liver disease, defined as:
   a. Aspartate transaminase (AST), alanine transaminase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >2 × ULN, or
   b. Baseline prothrombin time (international normalized ratio [INR]) >1.5 × ULN, or
   c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography (MRE).
3. Complete blood count: White blood cell <2,500 cells/μL; hemoglobin <11 g/dL for males, <10 g/dL for females; or platelet count <100,000/μL.
4. Baseline estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease (MDRD) equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick.
6. Inadequate diabetes control, with glycosylated hemoglobin >9%.
7. History of alcohol or drug abuse.
8. History of a significant coagulation disorder.

9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.1 mIU/L or >10 mIU/L).
10. Cardiac left ventricular ejection fraction <50% by echocardiogram.
11. Peripheral pulse oximetry saturation of <90%.
12. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic of >90 mmHg, confirmed by a repeat measurement.
13. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
14. Acute coronary syndrome event within 24 weeks prior to Day 1.
15. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
16. Acute pancreatitis within 12 weeks prior to Day 1.
17. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
18. Current use or use within 90 days from Day 1 of any monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab).
19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of selective serotonin reuptake inhibitors, chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of niacin-based supplements or nutraceuticals that may influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for human immunodeficiency virus type 1 (HIV-1) or HIV-2, hepatitis B virus (hepatitis B core antibody or nucleic acid testing [NAT]), or hepatitis C virus (NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the participant. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, has an intact uterus and at least 1 ovary, and is less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the participant would not comply with the study procedures outlined in the protocol.

Statistical Methods

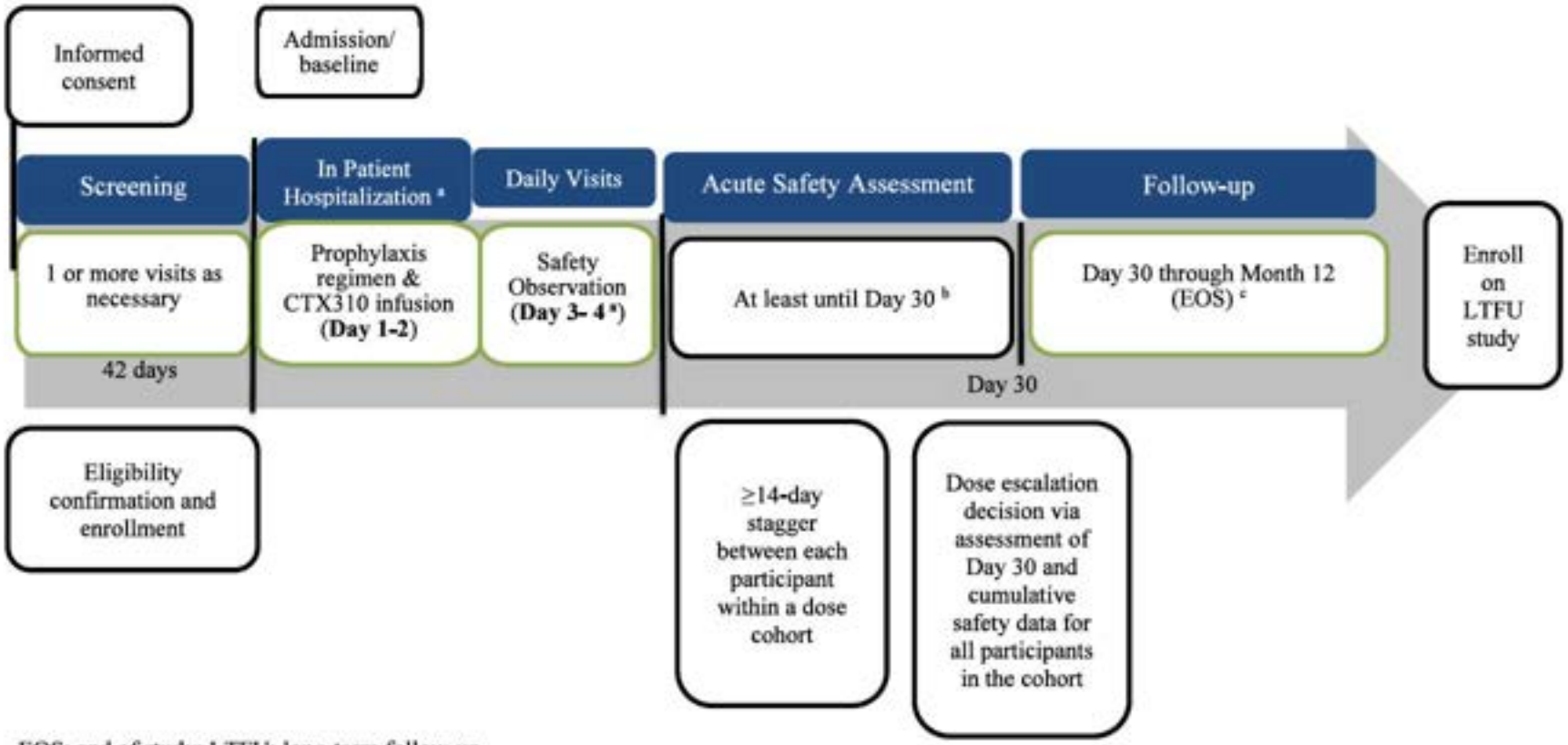
The study will initially enroll approximately 24 participants to provide a preliminary evaluation of safety and efficacy of CTX310.

Analysis populations are described in the statistical analyses section of the protocol. The safety and tolerability of CTX310 will be assessed in the safety analysis set using descriptive summaries. Summaries of AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each dose level of CTX310 and overall. Summaries of AEs will focus on treatment-emergent AEs (TEAEs). The incidence of TEAEs will be summarized by system organ class and preferred term, protocol-specified severity grade, and relation to CTX310. The incidence of DLTs, serious adverse events, and AESIs will be also summarized. Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of clinically significant laboratory abnormalities and other clinically significant safety measure abnormalities (e.g., ECG) will be summarized.

The preliminary efficacy of CTX310 will be assessed in the full analysis set using descriptive summaries. The percentage changes in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each dose level. Categorical summaries based on appropriate cutoff at selected time points, including 26 and 52 weeks after infusion, may be provided.

PK and PD data will be assessed descriptively and by exploratory modeling, as applicable.

1.2. Study Schema



EOS: end of study; LTFU: long-term follow-up.

[a] Inpatient hospitalization for CTX310 infusion on Day 1. Hospital discharge on Day 2 after the completion of safety evaluation and laboratory tests and a minimum of 24 hours after the completion of the CTX310 infusion. Daily safety visits on Day 3 and 4. Inpatient hospitalization may be extended, or the participant may be readmitted or additional daily safety visits beyond Day 4 may be required at the discretion of the investigator as needed for safety monitoring.

[b] For each cohort, there will be a safety monitoring period of ≥14 days between the treatment of each participant and any subsequent participants within the cohort, or until the participant is clinically stable and all laboratory values (including liver function tests) have returned to <2 × baseline or to normal levels, whichever is later.

[c] Consent for LTFU study should be obtained during the parent study EOS visit.

1.3. Schedule of Assessments

Table 1: Schedule of Assessments

		Treatment		Follow-up										
	Screening[1]	Inpatient Hospitalization		Daily Safety Visits										
Assessment	D -42 to -1	D1[2]	D2	D3	D4	W1 D7 ±1 d	W2 D14 ±2 d	W3 D21 ±4 d	D30 ±4 d	M2 ±7 d	M3 ±7 d	M6 ±7 d	M9 ±14 d	EOS M12 ±14 d[3]
Eligibility Confirmation (Also See Laboratory Assessments)														
Informed consent	X													
Demographics, medical history	X													
Physical exam[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height, weight, BMI, waist to hip ratio	X													X
Vital signs[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver MRE or FibroScan[6]	X													
Liver MRI-PDFF or ultrasound	X													X
Echocardiogram[7]	X								X					X
12-lead ECG[8]	X	X	X			X			X		X	X		X
Eligibility confirmation[9]	X													
Treatment														
Pre-infusion prophylaxis regimen[10]		X												
CTX310 infusion[11]		X												

		Treatment	Follow-up											
	Screening[1]	Inpatient Hospitalization		Daily Safety Visits										
Assessment	D -42 to -1	D1[2]	D2	D3	D4	W1 D7 ±1 d	W2 D14 ±2 d	W3 D21 ±4 d	D30 ±4 d	M2 ±7 d	M3 ±7 d	M6 ±7 d	M9 ±14 d	EOS M12 ±14 d[3]
Safety Assessments														
Acute DLT assessment		X	X	X	X	X	X	X	X					
Adverse events	X													
Concomitant meds	X													
Laboratory Assessment (Local) [12]														
Serum chemistry	X	X	X	X	X	X	X	X	X		X	X	X	X
Urinalysis	X		X		X				X		X	X	X	X
Coagulation panel	X	X	X	X	X	X	X	X	X		X	X	X	X
Pregnancy test[13]	X	X												X
Hematology (CBC)	X		X		X				X			X		X
HbA1c	X											X	X	X
Thyroid function	X											X		X
eGFR (MDRD equation)	X								X			X		X
Viral serology	X													
Laboratory Assessments (Central)[12]														
Genetic testing[14]	X													
Lipid panel[15]	X						X		X	X	X	X	X	X
Biomarkers (Plasma, Central)[16]														
ANGPTL3 levels[17]	X						X		X		X	X	X	X
PK studies[18]	X	X	X	X	X	X	X		X		X	X		X

		Treatment				Follow-up								
	Screening[1]	Inpatient Hospitalization		Daily Safety Visits										
Assessment	D -42 to -1	D1[2]	D2	D3	D4	W1 D7 ±1 d	W2 D14 ±2 d	W3 D21 ±4 d	D30 ±4 d	M2 ±7 d	M3 ±7 d	M6 ±7 d	M9 ±14 d	EOS M12 ±14 d[3]
Exploratory Biomarkers (Central)														
Immunogenicity[19]	X					X			X		X	X		X
Whole blood for storage[20]	X													
Plasma for storage[21]	X													
Serum[22]	X	X	X	X										

AESI: adverse event of special interest; ANGPTL3: angiopoietin-like 3; BMI: body mass index; Cas9: CRISPR-associated protein; CBC: complete blood count; CRISPR: clustered regularly interspaced short palindromic repeats; D or d: day; DLT: dose-limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; EOS: end of study; HbA1c: glycosylated hemoglobin; eLBW: estimated lean body weight; M: month; meds: medications; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI-PDFF: magnetic resonance imaging–protein density fat fraction; PK: pharmacokinetic; W: week

1 See Section 8.1.1.1 for detailed guidance.

2 Hospitalization: Participants will be hospitalized on Day 1 for CTX310 infusion. Hospital discharge will occur a minimum of 24 hours post completion of CTX310 infusion and after Day 2 safety evaluations are complete and relevant laboratory tests have been reviewed. Participants must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours post completion of CTX310 infusion, or the participant may be readmitted or required to stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring. See Section 6.1.2 for discharge criteria and Section 8.1.1 for further description of study periods.

3 All participants who received an infusion of CTX310, including those who terminate the study prior to 12 months, will be asked at the EOS visit to sign an informed consent for rollover into a separate long-term follow-up study for up to 15 years post-infusion (Section 8.1.1.5).

4 Complete physical exam required at screening, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3).

5 Vital signs: Blood pressure, heart rate, respiratory rate, oxygen saturation, temperature (Section 8.2.4). Participants excluded from the study due to uncontrolled hypertension must have blood pressure measurements repeated at least 15 minutes later for confirmation. On Day 1 vital signs should be recorded at the following timepoints: prior to the infusion of CTX310, every 15 minutes during the infusion, at 1, 2, and 5 hours after the end of the infusion, and then every 8 hours until discharge from the hospital.

6 Liver imaging: The same type of imaging should be used across all study visits (Section 8.2.5).

7 Echocardiogram: See Section 8.2.6.

8 ECG: See Section 8.2.7.

[9] Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment. Females of childbearing potential must be excluded from the study. Females with a uterus must be postmenopausal, defined by at least 12 consecutive months of amenorrhea without an alternative medical cause prior to screening, **or** surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, or oophorectomy at least 1 month prior to screening).

[10] Pre-infusion prophylaxis regimen: See Section 6.1.1.

[11] See Appendix 14.1 for eLBW calculation.

[12] See listings of laboratory assessments (Table 4 and Table 5) for details. On Day 1 laboratory values should be performed prior to the infusion of CTX310. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to define AESI (Section 9.3) and stopping rules (Section 10.2).

[13] All females enrolled in the study must have a negative pregnancy test within 72 hours prior to CTX310 infusion (Section 8.2.8, Table 4 and Section 8.2.9).

[14] The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.8 and Table 5 for details.

[15] Lipid panel: See Section 8.2.8, Table 5 for listing of lipid panel components. Participants on apheresis should have lipid levels sampled within 5 days prior to the procedure (pre-apheresis sample on the day of apheresis is also adequate). All lipid panels must be performed after a minimum 8 hour fast.

[16] Sponsor may request discontinuation of sample collections. Continue sample collection for all listed time points until instructed otherwise by the sponsor.

[17] Plasma samples will be obtained to assess ANGPTL3 levels (Section 8.4.2).

[18] PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, a single sample will be collected (Section 8.4.1).

[19] Immunogenicity: See Section 8.3.

[20] Whole blood collection: Whole blood samples will be obtained at screening and stored (Section 8.4.3.1).

[21] Plasma for storage: See Section 8.4.3.2.

[22] Serum samples for exploratory biomarker assessments (e.g., cytokines): On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, a single sample will be collected (Section 8.4.3.3).

2. INTRODUCTION

CTX310 is being developed by CRISPR Therapeutics AG (sponsor) for the treatment of participants with refractory dyslipidemias with persistently elevated levels of TG and/or non–high-density lipoprotein cholesterol (HDL-C) and/or low-density lipoprotein cholesterol (LDL-C) and/or apolipoprotein B (ApoB). It is an in vivo gene editing therapy designed to target the gene for *ANGPTL3*.

This first-in-human (FIH) study will evaluate the safety and tolerability of CTX310 in high-risk adult participants with dyslipidemias refractory to available treatments.

2.1. Dyslipidemias

Dyslipidemias are among the most commonly detected and treated chronic conditions. They are characterized by abnormal levels of related lipoprotein species and abnormal serum levels of cholesterol, TG, or both. One of the most common clinical consequences of dyslipidemias is increased risk of atherosclerotic cardiovascular disease (ASCVD), which is associated with elevated levels of non–HDL-C (primarily LDL-C) and TG. With ASCVD remaining the leading cause of death and disability worldwide (Barquera et al., 2015), new treatment options to add to the current standard of care are clearly needed.

The management of dyslipidemias remain the cornerstone of cardiovascular disease (CVD) prevention. As reported by the American Heart Association (AHA) in 2021, 38% of adults (93.9 million) in the United States had total cholesterol levels ≥200 mg/dL from 2015 to 2018, with elevated levels of LDL-C (≥130 mg/dL) reported in 29% of adults from 2013 to 2016 (Virani et al., 2021a). Dyslipidemias involving elevated levels of LDL-C (hypercholesterolemia), TG (hypertriglyceridemia [HTG]), or both also contribute to CVD and its associated risks, including type 2 diabetes, chronic kidney disease, and nonalcoholic fatty liver disease (Vijayaraghavan, 2010; Yang et al., 2021). Additional clinical consequences associated with rare dyslipidemias such as severe elevations in TG include increased risk of pancreatitis.

The latest recommendations of Canadian, Australian, European, and American cardiological associations emphasize the role of increased levels of non–HDL-C and ApoB in evaluating the risk of CVD (Brett et al., 2021; Grundy et al., 2019; Mach et al., 2020; Pearson et al., 2021; Virani et al., 2021b; Watts et al., 2021), rather than LDL-C and TG. Non–HDL cholesterol (i.e., total cholesterol – HDL-C) is the composite of LDL, intermediate-density lipoprotein [IDL], very low-density lipoprotein (VLDL), and Lp(a). ApoB, the major structural protein in VLDL, IDL, LDL-C, and Lp(a), is a highly atherogenic lipoprotein due to its arterial retention, resulting in plaque buildup in arterial walls over time (Sniderman et al., 2019). Although there is typically a good correlation between LDL-C and ApoB in calculating CVD risk, there is a discordance between the 2 parameters in approximately 20% of cases.

Therefore, non–HDL-C (indirectly) and ApoB (directly) provide a more accurate assessment of the total concentration of atherogenic particles, especially at higher TG concentrations, in non-fasting samples and in individuals with low LDL-C (Bergmann, 2010; Carr et al., 2019). The 2021 Canadian Cardiovascular Society (CCS) guidelines use either non–HDL-C or ApoB as the preferred parameter for assessment of CVD risk. Achievement of treatment target values for

ApoB and non–HDL-C have been modified from previous versions of CCS guidelines to accurately represent the same percentile equivalents as LDL-C for all recommended thresholds (Table 2) and inform the inclusion of non–HDL-C and ApoB in this study.

Table 2: ApoB and Non–HDL-C Threshold Selection

LDL-C		non–HDL-C		ApoB		
mmol/L	mg/dL	mmol/L	mg/dL	mmol/L	mg/dL	g/L
1.8	70	2.4	93	1.8	70	0.7
2.0	78	2.6	101	2.1	80	0.8
3.5	135	4.2	162	2.7	105	1.05
5	193	5.8	224	3.7	145	1.45

ApoB: apolipoprotein B; CCS: Canadian Cardiovascular Society; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.
Adapted from the 2021 CCS guidelines (Pearson et al., 2021).

Patients with dyslipidemias are typically treated with lipid-lowering therapies, which may include a statin, ezetimibe, lomitapide, icosapent ethyl, PCSK9 inhibitors (monoclonal antibodies and RNA inhibitor), and monoclonal antibody targeting ANGPTL3, where indicated and accessible. Despite all available treatments, only 45% of patients achieve target lipid levels suggested by AHA and American College of Cardiology (ACC) guidelines, especially patients who are at very high risk of cardiovascular events (Pearson et al., 2021; Rallidis et al., 2020), with approximately 50% of patients with ASCVD meeting the definition of high risk (An et al., 2020; Sajja et al., 2021).

Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on the following monogenic or polygenic refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes:

- Multifactorial chylomicronemia syndrome (MCS).
- Homozygous familial hypercholesterolemia (HoFH).
- Heterozygous familial hypercholesterolemia (HeFH).
- Other HTG and/or hypercholesterolemia syndromes of undetermined etiologies.

2.1.1. Hypertriglyceridemia

Per the Endocrine Society Clinical Practice Guidelines and the National Cholesterol Education Program Adult Treatment Panel III, normal fasting TG levels can be defined as <150 mg/dL, borderline high as 150 to 199 mg/dL, high as 200 to 499 mg/dL, and very high or severe as >500 mg/dL.

HTG is separated into 2 populations: secondary causes related to disease, medications, or diet, and primary genetic syndromes or susceptibility (Rygiel, 2018). Elevated TG levels (>150 mg/dL) are present in approximately 33% of adults in the US and are most commonly due to secondary causes rather than primary genetic syndromes (Oh and Trivette, 2020).

Acquired causes of HTG are most commonly due to medical conditions (e.g., diabetes mellitus, metabolic syndrome, central obesity, hypothyroidism, chronic kidney disease, autoimmune disorders), medications, and diet/lifestyle (e.g., alcohol use, physical activity; (Rygiel, 2018).

Genetic causes of HTG may be characterized as MCS and familial chylomicronemia syndrome (FCS).

MCS is a polygenic condition caused by heterozygous mutations in the lipoprotein lipase gene (*LPL*) or in the genes for apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*), lipase maturation factor 1 (*LMF-1*), or glycosylphosphatidylinositol anchored HDL binding protein 1 (*GP1HBP1*), or by multiple variants that are expressed in the presence of secondary factors (D'Erasmo et al., 2019). MCS is associated with a high CVD risk (Sarwar et al., 2007; Virani et al., 2021b) and affects approximately 1 in 250 to 1 in 800 persons (Fan et al., 2020; Laufs et al., 2020; Paquette and Bernard, 2022). TG levels range from the upper level of normal to severe (>150 to 1999 mg/dL).

FCS is a rare monogenic condition caused by the same homozygous or compound heterozygous mutations observed in MCS. Patients with FCS present at an earlier age, have severe levels of fasting TG and a higher incidence of acute pancreatitis. FCS is diagnosed based on fasting TG levels of ≥750 mg/dL that do not respond to standard lipid-lowering therapy (Brahm and Hegele, 2015). As most FCS patients are either LPL-deficient or lack insufficient LPL activity, these patients will likely not benefit from *ANGPTL3*-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and are, therefore, excluded from this study.

The effect of LDL-lowering drugs such as statins, ezetimibe and PCSK9 inhibitors on TG levels in patients with MCS is usually modest (5% to 15% improvement). The main goal of treatment of MCS is to reduce TG concentration to <5.6 mmol/L (500 mg/dL) in order to prevent acute pancreatitis (Christian et al., 2012; Scherer et al., 2014) followed by a secondary goal of reducing cardiovascular risk. The primary management of HTG consists of lifestyle modifications such as dietary fat restriction and reduced alcohol consumption, with the use of TG-lowering medications such as fibrates, omega-3 fatty acids, niacin, and icosapent ethyl, which can lower TG levels by 25% to 45% with limited impact on reductions in pancreatitis or CVD risk (Grundy et al., 2019). Intervention to prevent HTG in ASCVD is currently limited to icosapent ethyl, which was 25% effective at reducing relative risk for its primary study endpoint - a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, and unstable angina (Bhatt et al., 2019; Virani et al., 2021b).

Given the need for a significant improvement in TG-lowering therapies, based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of both LDL-C and TG.

2.1.2. Familial Hypercholesterolemia

Familial hypercholesterolemia is characterized by lifelong elevations in LDL-C and can be separated into 2 forms: HoFH and HeFH.

HoFH is a rare monogenic autosomal disorder with a prevalence of 1:300,000, and is characterized by significant elevations in LDL-C (Raal et al., 2020). Patients with HoFH develop tendon xanthomas (lipid deposits) and can develop premature CVD, including heart attack and

aortic valve disease, when they are teenagers or in their 20s, and many do not improve with cholesterol-lowering drugs. Without aggressive treatment, patients may die before age 30. The majority of HoFH (85% to 90%) is due to a biallelic deficiency or defect in the LDL receptor gene (*LDLR*). The remaining population have a defect in *ApoB* (loss-of-function [LOF] mutation) or *PCSK-9* (gain-of-function [GOF]; (Sjouke et al., 2015)). LDL-C levels are elevated due to a failure to synthesize LDLR (receptor-negative), or defective binding or release at the lipoprotein receptor interface, resulting in the inability to clear LDL-C from circulation and giving rise to LDL-C levels of >400 mg/dL. Traditional lipid-lowering therapies, including high-dose statins, PCSK9 inhibitors, bile acid sequestrants, and ezetimibe, have little to modest activity in HoFH. Plasmapheresis is used when available. The recent approval of an ANGPTL3-targeting antibody, evinacumab, provides a safe and effective option for patients with HoFH (Raal et al., 2020). When administered at 15 mg/kg on a monthly regimen, study subjects showed a 47% reduction in LDL-C compared to baseline, leaving significant room for improvement for LDL-lowering in this patient population. AHA guidelines recommend LDL-C levels <70 mg/dL in patients at high risk for CVD. Compared with repeat dosing of a monoclonal antibody, CTX310 may offer a one-time treatment option for HoFH patients who require additional and or significant LDL-C lowering.

HeFH is a common autosomal dominant disease, affecting approximately 1 in 250 people, with a significantly higher incidence in high-risk ASCVD (1:17) compared to the general population (Sturm et al., 2018). Similar to HoFH, the most common causes of HeFH are pathogenic variants of *LDLR*, which are responsible for 85% to 90% of genetically confirmed HeFH (Benn et al., 2016). Additional pathogenic variants of *ApoB* resulting in decreased binding of LDL to the LDLR, or GOF mutations in *PCSK9* result in increased destruction of LDLR and a >20-fold increase in ASCVD (Soutar and Naoumova, 2007). Although statins lower LDL-C by 18% to 55%, as many as 80% of statin-treated patients with established ASCVD fail to reach guideline-recommended target LDL-C levels (Marz et al., 2018). The addition of ezetimibe and PCSK9 inhibitors such as monoclonal antibodies and RNA inhibitors (inclisiran) to high-dose statin treatment lowers LDL-C by an additional ~15% to 50%, respectively (Grundy et al., 2019; Wright et al., 2021). Patients who are the least responsive to treatment are at highest risk of developing ASCVD. Evinacumab was evaluated in subjects with refractory hypercholesterolemia (LDL-C ≥100 mg/dL, or LDL-C ≥70 mg/dL with ASCVD; (Rosenson et al., 2020)). In this Phase 2 study, 70% to 80% of participants had HeFH, approximately 60% were receiving statin therapy, 100% were receiving a PCSK9 inhibitor, and approximately 30% were receiving ezetimibe, reflecting a population of patients with refractory hypercholesterolemia who may benefit from additional therapies designed to lower LDL-C. A reduction of LDL-C of up to 50% was achieved upon repeat dosing.

2.2. ANGPTL3

ANGPTL3 regulates plasma TG levels by inhibiting LPL, which is the key enzyme responsible for the breakdown or hydrolysis of TG into free fatty acids (FFA) and glycerol. ANGPTL3 also inhibits endothelial lipase (EL), which is primarily responsible for the lipolysis of HDL-C. In addition to its role in regulating TG levels, mice and humans with mutant *ANGPTL3* or inactivation of *ANGPTL3* rapidly clear VLDL in an independent manner (Adam et al., 2020; Musunuru et al., 2010; Shimizugawa et al., 2002; Wang et al., 2015). VLDL is a precursor

byproduct for the production or metabolism of LDL-C, hence its clearance limits the production of LDL-C. Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Dewey et al., 2017). ANGPTL3 inhibition also substantially lowers ApoB levels, which has been shown to proportionally decrease CVD risk (Ference et al., 2019).

Loss-of-function (LOF) variants in *ANGPTL3* have been associated with low levels of both LDL-C and TG, and a 41% lower risk of coronary artery disease, with no pathologic manifestations despite the presence of low levels of HDL-C (Athyros et al., 2018; Calandra et al., 2017; Dewey et al., 2017; Stitziel et al., 2017; Tarugi et al., 2019).

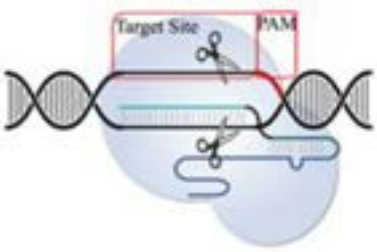
Collectively, these data indicate that ANGPTL3 is a valid therapeutic target to lower plasma LDL-C, non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who are at high risk of CVD. In vivo disruption of *ANGPTL3* in the liver using CTX310 may therefore provide clinical benefit in the study population with elevated lipid profiles selected for the FIH Phase 1 study.

2.3. CRISPR Technology

CRISPR (clustered regularly interspaced short palindromic repeats) are found flanking foreign DNA sequences in many bacteria and archaea. CRISPR are an important part of an adaptive bacterial defense system using RNA-guided DNA cleaving enzymes (Barrangou et al., 2007; Hale et al., 2009). The RNAs expressed from CRISPR sequences direct the sequence-specific binding of CRISPR-associated protein 9 (Cas9) nuclease. These key bacterial defense systems were adapted as a programmable RNA-directed CRISPR-Cas9 system for editing genomes (Jinek et al., 2012).

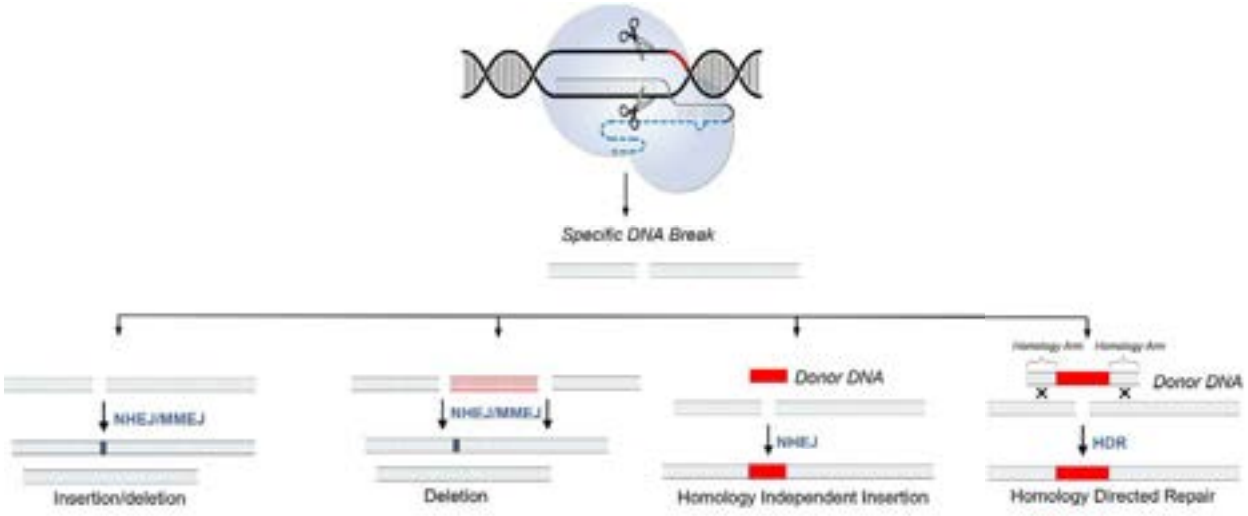
CRISPR-Cas9 systems can be directed by a complex of 2 distinct RNAs: crisprRNA (crRNA) and trans-activating crRNA (tracrRNA), or by a single-guide RNA (sgRNA) containing the crRNA and tracrRNA joined by a loop (Jinek et al., 2012). Delivery of Cas9 nuclease and sgRNA into a cell result in cleavage of the cell's genomic DNA at sequences specified by the sgRNA (Figure 1), leaving double-stranded breaks (DSBs). These DSBs are repaired by the cell's own DNA repair machinery. Nonhomologous end joining (NHEJ) is the predominant cellular repair pathway that is active during all phases of the cell cycle (Figure 2). However, NHEJ is an imprecise mechanism for DNA repair and often results in insertions or deletions (indels) at the cut site that can lead to gene disruption and potential LOF. The NHEJ repair pathway can be co-opted to insert DNA sequences at targeted Cas9-sgRNA cut sites in nondividing cells, a process referred to as homology-independent insertion (Figure 2). Homology directed repair (HDR) is the second most common repair mechanism for DSBs but, unlike NHEJ, is only active during late S and G2 phases of the cell cycle. HDR relies on the presence of a homologous repair template (Figure 2) and, as a result, DNA is often repaired faithfully with no indel formation. In cycling cells, HDR in the presence of genomic sequences containing homology arms can be used to correct mutations or introduce novel sequences at specific cut sites.

Figure 1: Schematic of the CRISPR-Cas9 Complex



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; crRNA: crisprRNA; PAM: protospacer adjacent motif; tracrRNA: trans-activating crRNA.
CRISPR-Cas9 complex containing a single-guide RNA wherein the crRNA and tracrRNA are joined by a linker loop.

Figure 2: CRISPR-Cas9–mediated Genome Editing Strategies



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; MMEJ: microhomology-mediated end joining; NHEJ: nonhomologous end joining.

Target sites for CRISPR-Cas9 systems are distributed throughout the genome. A requirement is that the target sequence, homologous to the 5' end of the sgRNA and typically 20 nucleotides long, is followed by a protospacer adjacent motif (PAM) sequence (Horvath et al., 2008; Mojica et al., 2009; Shah et al., 2013). For *Streptococcus pyogenes* (Sp) Cas9, the PAM is any nucleotide followed by a pair of guanines (denoted as NGG).

The Cas9 nuclease searches for the PAM site and adjacent sequence matching the sgRNA 5' end before cleaving the DNA. Target site specificity results from a required combination of the site matching the sgRNA adjacent to a PAM site: the Cas9 nuclease does not bind to sequences without being complexed to a matching sgRNA, and the Cas9 nuclease and sgRNA will not bind or cut unless the target sequence is adjacent to the PAM.

CTX310 utilizes CRISPR-Cas9 to selectively cut exon 1 of *ANGPTL3*, which is specifically expressed in the liver. The resulting indels via NHEJ-mediated repair (active in nondividing

hepatocytes) lead to small frameshift mutations and a premature stop codon, resulting in protein knockdown. The consequence of the gene editing is the reduction of ANGPTL3 protein secreted into circulation.

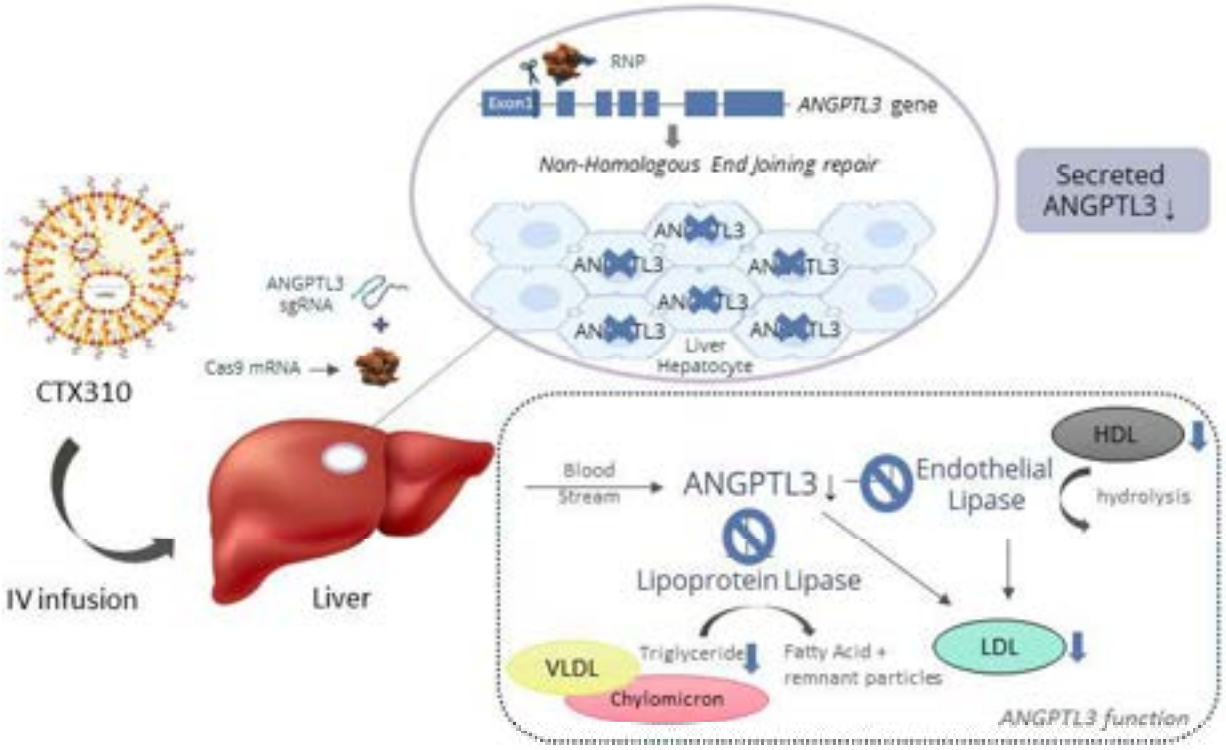
2.4. CTX310

CTX310 is a lipid nanoparticle (LNP) formulation of CRISPR-Cas9 components for in vivo gene editing of the target gene *ANGPTL3*.

The CTX310 drug product (DP) is a sterile formulation that consists of 2 drug substances (DSs): messenger RNA (mRNA) encoding SpCas9, and [redacted] sgRNA targeting the gene of interest with a defined mass ratio encapsulated in an LNP, both of which will be individually manufactured prior to coformulation into DP. The DSs and DP will be manufactured and stored according to Good Manufacturing Practice. The first DS is a capped and polyadenylated SpCas9 mRNA containing N1-methylpseudouridine. The second DS, sgRNA, is a 100 nucleotide–long single-stranded oligonucleotide. The DP is an LNP encapsulating the 2 DSs and is composed of 4 lipid components: a cationic lipid, a polyethylene glycol lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, and cholesterol. The DP LNPs have an average size of ~60 nm and range in size from approximately 50 to 100 nm.

The mechanism of action for CTX310 is the disruption and reduction of the ANGPTL3 biological pathway (Figure 3). Nonclinical data supporting the clinical use of CTX310 are summarized in the Investigator Brochure.

Figure 3: CTX310 Mechanism of Action



ANGPTL3: angiopoietin-like 3; Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; EL: endothelial lipase; HDL: high-density lipoprotein; IV: intravenous; LDL: low-density lipoprotein; LNP: lipid nanoparticle; LPL: lipoprotein lipase; mRNA: messenger RNA; NHEJ: nonhomologous end joining; RNA: ribonucleic acid; RNP: ribonucleoprotein; sgRNA: single-guide RNA; SpCas9: *Streptococcus pyogenes* CRISPR-associated protein 9; TG: triglyceride; VLDL: very low-density lipoprotein.
CTX310 is delivered via IV infusion. Similar to other LNPs, CTX310 is expected to be taken up by liver hepatocytes. After uptake, CTX310 escapes from endosomes and releases encapsulated SpCas9 mRNA and sgRNA into cytosol. SpCas9 mRNA is translated to SpCas9 protein, which then forms an RNP complex with sgRNA; this RNP complex shuttles into the nucleus and binds the target sequence. The RNP complex cuts at exon 1 of the *ANGPTL3* locus and introduces frameshift mutations after NHEJ repair. This leads to the knockdown of ANGPTL3 protein expression and reduced secretion from hepatocytes into circulation. The dotted box portrays the role of ANGPTL3 in lipoprotein metabolism. ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; EL hydrolyzes HDL. ANGPTL3 also affects LDL levels through LDL receptor–dependent and –independent (via EL) pathways. Knockdown of ANGPTL3 de-represses the activity of these lipases and leads to reduction of TG and LDL levels.

2.5. Study Rationale

A one-time in vivo disruption of *ANGPTL3* in the liver using CTX310 may provide clinical benefit in patients with dyslipidemia with elevated levels of TG and/or non–HDL-C (including LDL-C) and/or ApoB that are refractory to current treatments, and where compliance to adherence with lifelong medications and optimal lifestyle continue to remain a challenge.

2.5.1. Rationale for Targeting *ANGPTL3*

The sponsor is developing a one-time gene editing therapy for patients with dyslipidemias who have responded inadequately to maximum tolerated doses (MTDs) and adequate duration of currently available treatments and have not achieved the target lipid levels recommended by current guidelines (i.e., who are refractory). The gene editing therapy utilizes CRISPR-Cas9 to specifically target and disrupt *ANGPTL3*, which encodes a regulator of lipoprotein metabolism expressed in the liver (Conklin et al., 1999) and has emerged as a therapeutic target for patients with mixed dyslipidemias. As described in Section 2.2, ANGPTL3 has been shown to inhibit activity of LPL, the main enzyme involved in hydrolysis of TG-rich lipoproteins, and EL, which hydrolyzes HDL phospholipids, and therefore increases TG and other lipids (Kersten, 2021; Shimamura et al., 2007; Shimizugawa et al., 2002). Decreased ANGPTL3 levels have been shown to exhibit higher LPL activity and thus reduced levels of TG (Christopoulou et al., 2019). *ANGPTL3* inhibition can also lead to efficient clearance of VLDL particles via activation of EL in an LDLR-independent mechanism, leading to reduction in LDL-C, non–HDL-C, and ApoB levels (Adam et al., 2020; Rosenson et al., 2020).

Large-scale genetic studies in humans show LOF variants of *ANGPTL3* have low levels of TG and LDL-C and decreased risk of ASCVD (Dewey et al., 2017; Helgadottir et al., 2016) despite low levels of HDL-C (Minicocci et al., 2012; Musunuru et al., 2010; Stitziel et al., 2017). In addition, clinical studies targeting *ANGPTL3* by lowering or inactivating through antisense oligonucleotide or monoclonal antibody treatments have demonstrated efficacy in subjects with various forms of dyslipidemia to markedly reduce plasma LDL-C and TG levels (Graham et al., 2017; Raal et al., 2020; Rosenson et al., 2020; Watts et al., 2019). *ANGPTL3* inhibition also substantially lowers ApoB levels, which has been shown to proportionally decrease risk of CVD (Ference et al., 2019). Together, these studies indicate that *ANGPTL3* is a valid therapeutic target to lower plasma non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who remain at high risk of CVD.

2.5.2. Rationale for the Study Population

Based on the preclinical understanding of and emerging clinical data from ANGPTL3-directed therapies, and a high unmet need in a treatment-refractory population at high risk for cardiovascular events, this Phase 1 study will include participants with or without ASCVD who have monogenic or polygenic refractory dyslipidemias, including MCS, HoFH, HeFH, and other HTG/hypercholesterolemia syndromes of undetermined etiologies (Section 2.1).

Based on data from non–GLP and GLP toxicology studies in non-human primate (NHP) models in which non-adverse, dose-dependent transient elevations in liver function tests (LFTs) were observed, eligibility criteria for the clinical study will exclude participants with underlying liver fibrosis or past infections, alcohol abuse, compromised liver function, or co-morbidities that may compromise liver function. As the liver is the main target organ for CTX310, participants with coagulation disorders or low platelet counts are also excluded. Participants who do not meet adequate organ function criteria (e.g., for renal, lung, and cardiac function) are excluded from participating in the Phase 1 study.

As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema based on estimated lean body weight (eLBW), which takes into account gender and height rather than total body weight alone, was selected to reduce the risk of overdosing participants with higher body mass index (BMI).

Due to the unknown risk of on-target editing in tissues other than the liver, including reproductive organs, women of childbearing potential will be excluded from this study, and male participants enrolled in the study must agree to use highly effective method(s) of contraception from study consent through 12 months after CTX310 infusion, until additional nonclinical data are available to reassess the eligibility criteria (see Section 4).

The study considers the balance of risk and potential benefit for participants in this FIH study of CTX310.

2.5.3. Rationale for Lipid Cutoff Values for Inclusion

Per AHA and CCS guidelines (Grundy et al., 2019; Pearson et al., 2021), participants at high risk of CVD (i.e., LDL-C ≥100 mg/dL for participants without ASCVD or ≥70 mg/dL for participants with established ASCVD) are recommended for this study.

Similarly, participants with non–HDL-C levels of ≥160 mg/dL or ApoB levels of ≥100 mg/dL, identified as at high risk of CVD per CCS guidelines (Pearson et al., 2021), are also recommended for this study.

Although TG levels of ≥150 mg/dL are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al., 2020; Watts et al., 2021), a higher TG level (≥300 mg/dL) has been recommended for inclusion in this study to select for a more severely dyslipidemic patient population.

2.5.4. Preclinical Data

In NHP studies, a single dose of CTX310 resulted in significant and sustained reductions in TG levels in a dose-dependent manner, and in a mouse *LDLR* knockout model, a mouse surrogate of CTX310 resulted in significant lowering of LDL-C. Together, these preclinical data, summarized in the Investigator's Brochure, support the use of CTX310 as a one-time treatment to lower atherogenic lipids.

3. STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of AEs, including TEAEs, AESIs, DLTs; clinically significant laboratory abnormalities; and clinically significant abnormal vital signs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline
Exploratory Objectives	**Exploratory Endpoints**
• To identify changes associated with CTX310 that may indicate or predict clinical response, immunogenicity, safety, or PD activity	• Percentage change in FFA levels over time compared to baseline. • Change in fatty liver disease. • Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required).

ADA: anti-drug antibody; AE: adverse event; AESI: adverse event of special interest; [redacted]

ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; DLT: dose-limiting toxicity; FFA: free fatty acid; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; Lp(a): lipoprotein(a); PD: pharmacodynamic(s); PEG: polyethylene glycol; PK: pharmacokinetics; TEAE: treatment-emergent adverse event; TG: triglycerides.

4. PARTICIPANT ELIGIBILITY

4.1. Inclusion Criteria

To be considered eligible to participate in this study, a participant must meet all the inclusion criteria listed below:

1. Age of ≥18 and ≤70 years at the time of signing informed consent.
2. Able to provide written informed consent.
3. Participants diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>300 mg/dL) and/or LDL-C (>100 mg/dL; >70 mg/dL for participants with ASCVD) and/or non–HDL-C (>160 mg/dL) and/or ApoB (>100 mg/dL) at screening.
4. Participants must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, icosapent ethyl, monoclonal antibodies to PCSK9 (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening.
5. Participants with HoFH receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Participants on available standard of care lines of treatment, including statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors, must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation.
7. Participants on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change in frequency during the study participation.
8. Female participants must be postmenopausal, defined as:
   - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
   - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male participants must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in Section 14.2 , from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.
11. Willing to participate in a long-term follow-up study for up to 15 years after completion of this study.

4.2. Exclusion Criteria

To be eligible for entry into the study, the participant must not meet any of the exclusion criteria listed below:

1. Participants with FCS as documented in the medical history.
2. Evidence of liver disease, defined as:
    a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >2 × ULN, or
    b. Baseline prothrombin time (INR) >1.5 × ULN, or
    c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography (MRE).
3. Complete blood count: White blood cell <2,500 cells/μL; hemoglobin <11 g/dL for males, <10 g/dL for females; or platelet count <100,000/μL.
4. Baseline estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick.
6. Inadequate diabetes control, with glycosylated hemoglobin >9%.
7. History of alcohol or drug abuse.
8. History of a significant coagulation disorder.
9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.1 mIU/L or >10 mIU/L).
10. Cardiac left ventricular ejection fraction <50% by echocardiogram.
11. Peripheral pulse oximetry saturation of <90%.
12. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic >90 mmHg confirmed by a repeat measurement.
13. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
14. Acute coronary syndrome event within 24 weeks prior to Day 1.
15. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
16. Acute pancreatitis within 12 weeks prior to Day 1.
17. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
18. Current use or use within 90 days from Day 1 of any monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab).

19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of selective serotonin reuptake inhibitors, chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of niacin-based supplements or nutraceuticals that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for HIV-1 or HIV-2, hepatitis B virus (hepatitis B core antibody or NAT), or hepatitis C virus (NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the participant. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, has an intact uterus and at least 1 ovary, and is less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the participant would not comply with the study procedures outlined in the protocol.

5. STUDY DESIGN

5.1. Investigational Plan

This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll approximately 24 participants 18 to 70 years (inclusive) of age who have dyslipidemias with persistently elevated levels of TG and/or non–HDL-C [including LDL, VLDL, IDL, and Lp(a)] and/or ApoB above the thresholds recommended by ACC and AHA guidelines despite MTDs of available lipid-lowering treatments, diet, and lifestyle modifications (i.e., refractory to indicated and available treatments; see Inclusion Criteria in Section 4.1).

This Phase 1 study will include participants with monogenic or polygenic refractory dyslipidemias, with or without ASCVD, that encompass HTG and/or hypercholesterolemia syndromes, as described in Section 2.5.2.

The majority of participants enrolled are expected to have HeFH or be of polygenic background due to the high prevalence of HeFH and polygenic hypercholesterolemia and HTG (Goldberg and Chait, 2020; McGowan et al., 2019; Sturm et al., 2018). Participants with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing CVD risk. Participants will be asked to continue to take their baseline lipid-lowering medications in the same doses throughout the study; adequate washout periods between the medications and CTX310 infusion have been included, when relevant.

Three to 6 participants will be enrolled in each of the dose levels: 0.1, 0.3, 0.6, and 0.8 mg/kg eLBW of total RNA in the LNP formulation. Within each dose level, a minimum 14-day stagger between dosing of CTX310 to each participant is required. Dose escalation will follow the criteria described in Section 5.4.

Each participant will receive a single IV dose of CTX310 on Day 1 and will be hospitalized for a minimum of 24 hours after completion of CTX310 infusion (or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment). following hospital discharge, participants may be readmitted, if needed, safety assessment. all participants will be closely monitored post-infusion for adverse events (AEs) defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All participants will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Participants will be required to stay within 1 hour of the infusion site to enable daily (and as needed) clinic visits for safety assessment until completion of Day 4 visit (or longer at the discretion of the investigator for safety assessment).

After CTX310 infusion, participants will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on ANGPTL3 levels and lipid profile. After completion of this study, all participants will be asked to participate in a separate long-term follow-up study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events.

At each dose level, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next cohort. Once dose escalation is completed and a RP2D has been determined, at least 3 additional participants

will be enrolled at the same dose level to confirm safety and pharmacodynamic (PD) effect (confirmatory cohort).

5.2. Number of Study Participants

Approximately 24 eligible participants will be enrolled in the study.

5.3. Study Duration

As illustrated in the study schema (Section 1.2), each participant will undergo the following stages:

1. Screening: up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All participants will be monitored for safety, tolerability, pharmacokinetic (PK), and PD effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: All participants will be asked to roll over to a separate long-term follow-up study (Section 8.1.1.5).

5.4. Dose Escalation

5.4.1. Dose Escalation Methodology

The doses in Table 3 are proposed for evaluation in the 4 planned dose escalation levels in the study, with a minimum of 3 and a maximum of 6 evaluable participants per DL.

Table 3: Dose Escalation of CTX310

Dose Level	Planned Dose (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6 [2]
4	0.8 [3]

DL: Dose Level; eLBW: estimated lean body weight.

[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW. See Appendix 14.1 for eLBW calculation.

[2] Following review of clinical data by the sponsor and Safety Review Committee at DL3, a de-escalation to a dose of 0.5 mg/kg eLBW may be explored.

[3] Following review of clinical data by the Sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 participants will be treated at each dose level depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in LFTs after dosing with CTX310 resolved within 14 days, the dosing between each participant within a cohort will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs)

have returned to <2 × baseline or to normal levels, whichever is later. If the safety evaluation of a participant is acceptable, the next participant in the cohort may be dosed.

Dose escalation may proceed when all participants in the preceding dose cohort have completed dosing, the last participant has completed ≥30-day safety evaluation, and the cumulative safety data of all treated participants at that dose level demonstrates an acceptable safety profile, as determined by the SRC.

The SRC will review all available cumulative safety and clinical activity data when the DLT observation period ends for the last participant enrolled in each cohort and will be responsible for making dose escalation decisions. Throughout dose escalation, for cases in which a dose had been cleared in a cohort and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to enroll an additional number of participants for a total of up to 6 at the current dose level to gather additional safety data. Based on ongoing assessment of benefit and risk, the SRC may stop dose escalation before an MTD is determined.

The sponsor, in conjunction with the investigators and the SRC, will decide whether to classify an event occurring within the first 30 days following infusion as a DLT.

Dose escalation will be performed according to the following rules:

- If 0 of 3 participants experience a DLT, escalate to the next dose level.
- If 1 of 3 participants experiences a DLT, expand the current dose level to 6 participants.
  - If 1 of 6 participants experiences a DLT, escalate to the next dose level.
  - If ≥2 of 6 participants experience a DLT in DLs 2, 3, or 4, de-escalate to previous dose level, or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- If ≥2 of 3 participants experience a DLT in DLs 2, 3, or 4, or any optional de-escalation dose level as specified in Table 3, de-escalate to previous dose level or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- No dose escalation is planned beyond the highest dose level listed for the study (Table 3).

The sponsor will declare the RP2D at or below the MTD, or alternatively an optimum biological dose (OBD) based on the analysis of clinical data. At least 3 additional participants will be administered with CTX310 at this dose level (MTD or OBD) before an RP2D is confirmed. A dose expansion cohort may be added to the protocol in a future amendment.

Toxicities will be graded and documented per criteria described in Section 9.

All cumulative AEs occurring outside the acute DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the independent Data Safety Monitoring Board (DSMB).

5.4.2. Maximum Tolerated Dose/Optimum Biologic Dose Definition

The MTD is the highest dose for which DLTs are observed in fewer than 2 of 6 participants. An MTD may not be determined in this study. The OBD is the lowest dose associated with biological efficacy, as determined by intended decrease in ANGPTL3 levels, with minimum toxicity.

5.4.3. Dose-limiting Toxicity Rationale and Definitions

The DLT definitions used in this study are informed by nonclinical studies of CTX310, and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021). AEs that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

- Any CTCAE grade ≥3 AE that is related to study drug.
- Any CTCAE grade 3 laboratory abnormality that persists ≥7 days and is related to study drug.
- Any CTCAE grade 4 laboratory abnormality that is related to study drug.

Participants must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable participant (i.e., a participant that has been administered CTX310, and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable participants are required per cohort.

An adequate interval will be applied between current lipid-lowering treatments a participant is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities.

Note: Any participant who experiences a DLT will be considered evaluable. Data for all participants who receive CTX310 will be part of the safety analysis set.

5.5. CTX310 Dose Rationale

The FIH starting dose is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the NHP GLP safety and toxicology study. The initial starting dose of 0.1 mg/kg of CTX310 refers to the total RNA dose that is based on an anticipated NOAEL of 1 mg/kg. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg. The emerging clinical data on systemically infused LNP-associated therapeutics demonstrate a relatively safe profile (Adams et al., 2018; Coelho et al., 2013; Gillmore et al., 2021). A 3-fold safety factor is proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies (Barros and Gollob, 2012). With an anticipated 3- to 2-fold increment in dose levels, dose escalation is expected to proceed from 0.1 to 0.8 mg/kg. Dose escalation decisions will be made

in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data.

6. STUDY TREATMENT

6.1. Administration of CTX310

Participants will receive a single IV infusion within a 1-hour period on Day 1, administered under medical supervision during inpatient hospitalization. The date and time of the dose administered will be recorded in the source documents and in the electronic case report form (eCRF).

Infusion of the study drug will be slowed or stopped in the event of an infusion-related reaction (IRR; Section 7.1.1).

6.1.1. Pre-infusion Prophylaxis

Within 1 to 2 hours prior to the administration of study drug, an infusion prophylaxis regimen will be administered to participants. The regimen consists of the following:

- IV steroid (e.g., dexamethasone 10 mg or equivalent);
- IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent) or oral H1 blocker (e.g., cetirizine 10 mg or equivalent); and
- IV or oral H2 blocker (e.g., famotidine 20 mg or equivalent).

6.1.2. CTX310 Post-infusion Monitoring

Following completion of study drug administration, participants will be observed as inpatients for minimum of 24 hours, or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment. Participants must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours post completion of CTX310 infusion, or the participant may be readmitted or required to stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring.

Safety and clinical laboratory evaluations, and collection of blood and urine samples will be performed per the schedule of assessment (Table 1). Repeat laboratory evaluations may be required for assessment of AESI or to meet study stopping criteria (see Section 9.3 and Section 10.1). All AEs and concomitant medications will be recorded.

Inpatient hospitalization for observation may be extended at the discretion of the investigator to follow and manage AEs as needed.

Participants will be discharged from the study site when they meet the following criteria:

1. Are clinically stable as per the investigator's judgement.
2. Day 2 safety assessments have been performed and relevant laboratory results have been reviewed.
3. The frequency of monitoring of laboratory AEs can be handled in the outpatient setting (schedule of assessments Table 1 and Section 7.1.2).

4. Participants are aware of contact information in case of an emergency and agree to remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit.

6.2. Investigational Product Preparation, Handling, Storage, and Accountability

CTX310 DP will be provided as a frozen liquid formulation consisting of 300 mM sucrose in phosphate buffered saline at a target concentration of 2.0 (± 0.4) mg/mL total RNA. CTX310 must be stored frozen at ≤-60°C in a glass vial until time of use. The DP will be stored onsite, thawed, and formulated immediately prior to administration. Refer to the Pharmacy Manual for detailed instructions on preparation, storage, handling, and administration of CTX310.

6.2.1. Investigational Product Accountability

The investigator and sponsor are responsible for accountability and traceability of CTX310 clinical supply.

The investigator will ensure that CTX310 is used in accordance with this protocol and the Pharmacy Manual. Detailed accountability records indicating CTX310 inventory at each clinical site, use by each participant, and disposal will be maintained by the clinical sites. To maintain compliance, the sponsor or its designee will review CTX310 clinical supply accountability records at the clinical sites on an ongoing basis during monitoring visits.

Instructions for destruction of all excess and expired material containing CTX310 will be provided directly by the sponsor. Destruction will be adequately documented and reviewed regularly by the sponsor or its designee and the investigator.

6.3. Comparator Product

This is a single-arm study with no comparator.

6.4. Measures to Minimize Bias: Randomization and Blinding

This is a single-arm, open-label study. Masking is not applicable. Randomization is not used in this study.

6.5. Prior and Concomitant Medications

All medications taken within 30 days before the signing of the informed consent form (ICF) will be recorded. All concurrent therapies, including prescription and nonprescription medications, must be recorded from the date of signed informed consent through 12 months after CTX310 infusion.

6.5.1. Allowed Medications and Procedures

Topical, intra-articular, nasal, inhaled, and ophthalmic steroid therapies are not considered systemic and are allowed. Participants may continue medications or treatments deemed necessary by the investigators to provide adequate supportive treatment for optimal medical care

throughout the study, including previously prescribed medications for management hypertension and lipid levels except for the prohibited medications listed in Section 6.5.2.

The dose and regimen of TG- or LDL cholesterol-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, icosapent ethyl, evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study.

The following **dosing windows** surrounding the infusion of CTX310 are suggested for lipid-lowering therapies:

- Monoclonal antibodies (alirocumab, evolocumab, and evinacumab) may not be administered beginning 7 days before the infusion of CTX310 and until 7 days after Day 1.
- Inclisiran may not be administered beginning 60 days before infusion of CTX310 and until 60 days after Day 1.
- Apheresis procedures may not be performed beginning 14 days before infusion of CTX310 and until 14 days after Day 1.

If a significant lowering of lipids (LDL-C or TG) is observed during the course of the study, i.e., lipid levels decrease to the desired levels (e.g., LDL <70 mg/dL, TG <150 mg/dL, or non–HDL-C <160 mg/dL), adjustments to relevant medications or frequency of apheresis procedures may be instituted by the investigator after discussion with the sponsor. It is expected that the plan for tapering other lipid-lowering medications or apheresis procedures will be individualized for each participant by the investigator depending on the response to study treatment, underlying genotype, and assessment of risk factors for future cardiovascular events.

6.5.2. Prohibited Medications

Medications prohibited prior to enrolling in the study or administration of CTX310 are noted in the exclusion criteria (Section 4.2) and are as follows:

- Hepatocyte-targeted small interfering RNA or antisense oligonucleotide molecules (except inclisiran).
- Monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab).
- Any investigational product.
- Selective serotonin reuptake inhibitors.
- Chronic systemic corticosteroids.
- Anabolic agents.
- Niacin-based supplements or nutraceuticals that may influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
- Prior treatment with a gene therapy/editing product.

6.6. Lifestyle Considerations

For participants who consume alcohol the following recommendations are provided regarding alcohol intake:

- Abstain from alcohol for 2 weeks before and 2 weeks after CTX310 infusion.
- A maximum of 6 standard drinks per week but no more than 2 standard drinks per day for the duration of the study.

7. SAFETY MONITORING RULES

7.1. General Guidance

Participants will be closely monitored for DLTs for 30 days after CTX310 infusion. Investigators are required to proactively monitor and treat all AEs in accordance with protocol guidance.

Although this is an FIH study and the clinical safety profile of CTX310 has not been previously described, the following general recommendations are provided based on prior experience with an LNP-formulated CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021) and *ANGPTL3*-targeted antisense oligonucleotide or interfering RNA therapies (Graham et al., 2017; Watts et al., 2022).

The safety profile of CTX310 will be continually assessed throughout the study, and investigators will be updated on a regular basis with new information regarding the identification and management of potential CTX310-related toxicity (refer to Investigator's Brochure).

7.1.1. Infusion-related Reactions

In the event of an IRR, the infusion of study drug will be slowed or stopped, and the participant closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study drug administration include but are not limited to acetaminophen/paracetamol, H1/H2 blockers, nonsteroidal anti-inflammatory drugs, epinephrine, supplemental oxygen, IV fluids, and/or corticosteroids. Caution should be exercised in the use of acetominophen/paracetomol.

Following resolution of a mild or moderate IRR that required interruption or slowing of the study drug infusion, administration may resume or continue at the investigator's discretion at a slower infusion rate.

Study drug administration will not be resumed for any participant following a severe IRR until the case is discussed with the medical monitor.

7.1.2. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the FDA's 2009 draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation."

Any ALT or AST measurement that is >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) at any time during the study (treatment or post-treatment period), will be reported as AESI (Section 9.3) and the measurement(s) should be confirmed by repeat testing within 24 to 48 hours of all 4 of the usual serum measures (ALT, AST, alkaline phosphatase [AP], and total bilirubin) to confirm the abnormalities.

Participants with confirmed ALT or AST levels >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) should have liver chemistry tests (ALT, AST, AP, INR, and total bilirubin) retested at least twice weekly until ALT and AST levels become ≤1.2 × ULN, or 1.2 × baseline value if the baseline value was >ULN. In addition, the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases.

2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
3. Obtain a history for travel and exposure to environmental chemical agents.
4. Serology for viral hepatitis (hepatitis A virus immunoglobulin M [IgM], hepatitis B surface antigen, hepatitis C virus antibody, cytomegalovirus IgM, and Epstein-Barr antibody panel).
5. Serology for autoimmune hepatitis (e.g., antinuclear antibody).

Additional liver evaluations, including gastroenterology/hepatology consultations, or hepatic computed tomography or magnetic resonance imaging (MRI), may be performed at the discretion of the investigator in consultation with the sponsor medical monitor. Repetition of the above evaluations should be considered if a participant's ALT and/or AST levels reach 5 × ULN.

8. STUDY PROCEDURES

A complete schedule of assessments is provided in Table 1. Descriptions of all required study procedures are provided in this section. In addition to protocol-mandated assessments, participants should be followed per institutional guidelines, and unscheduled assessments should be performed when clinically indicated.

Missed evaluations should be rescheduled and performed as close to the originally scheduled date as possible except if rescheduling becomes, in the investigator's opinion, medically unnecessary or unsafe because it is too close in time to the next scheduled evaluation. In that case, the missed evaluation will be recorded as a protocol deviation and should be abandoned.

For the purposes of this protocol, there is no Day 0. All visit dates and windows are to be calculated using Day 1 as the date of CTX310 infusion.

8.1. Participant Screening, Enrollment, and Withdrawal

8.1.1. General Study Periods

8.1.1.1. Screening and Enrollment

Investigators will keep a log of all potential participants reviewed and evaluated for study participation. Enrolled participants are defined as participants who consent to participate in the clinical study and whose eligibility is confirmed (meet the inclusion/exclusion criteria). Screen failures are defined as participants who consent to participate in the clinical study but do not meet the eligibility criteria.

The screening period begins on the date that the participant signs the ICF and continues through confirmation of eligibility and enrollment into the study. Once informed consent has been obtained, the participants will be screened to confirm study eligibility, as outlined in the schedule of assessments (Table 1). All screening assessments should be completed within 42 days after a participant signs the ICF. The medical monitor will review eligibility packets and verify information provided by the site to confirm agreement with the investigator that the participant is eligible for enrollment.

Repetition of individual screening assessment(s) that did not meet eligibility requirements is not permitted with the following exceptions:

- If there is clear evidence of a laboratory error (e.g., hemolyzed sample) or equipment malfunction, collection of a repeat sample for the appropriate laboratory test or assessment may be permitted with the approval of the medical monitor.
- Individual laboratory results that, in the opinion of the investigator, are related to a temporary, reversible condition may be retested once after the condition resolves or within 7 days, whichever is earlier.

If repeat values of the individual assessment(s) are within the eligibility criteria and completed within the screening window, then the participant is eligible for the study.

Participants will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for

genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI/ultrasound of liver, echocardiogram, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

Specific procedures for enrollment will be provided to sites.

8.1.1.2. Infusion of CTX310

All participants will receive a pretreatment regimen and study treatment, as described in Section 6.1.

8.1.1.3. Acute Safety Evaluation Period

The acute safety evaluation period is 30 days following infusion of CTX310 for each participant.

8.1.1.4. Follow-up and End of Study Definition

Following the 30-day acute safety evaluation period, participants will be followed for an additional 11 months. Participants will be considered to have completed the study after they complete the end of study (EOS) visit at Month 12.

The end of the study is defined as the time at which the last participant completes the Month 12 visit, is considered lost to follow-up, withdraws consent, or dies.

8.1.1.5. Long-term Follow-up

To comply with local regulatory requirements/guidance for participants administered a gene therapy, all participants who receive an infusion of CTX310 and either discontinue or complete this study will be asked to participate in a separate long-term follow-up study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events.

8.1.2. Participant Identification

A unique participant number will be assigned when an individual participant signs the study ICF. Participants will be identified by a participant number consisting of:

> Last 3 digits of protocol number (400), assigned 3-digit site number, sequential 3-digit participant number (e.g., 400-XXX-YYY)

Once a number is assigned to a participant, it cannot be reassigned to a different participant. Rescreened participants will keep the same participant number assigned during the initial screening process.

8.1.3. Replacement of Participants

Participants must receive CTX310 to be evaluated for a DLT. If a participant discontinues the study at any time prior to CTX310 infusion, the participant will be deemed unevaluable for DLT and will be replaced. If a DLT-evaluable participant (i.e., a participant who has been administered CTX310) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared.

Participants who discontinue from the study at any other time post–CTX310 infusion for any other reason will not be replaced.

8.1.4. Participant Withdrawal or Discontinuation

Participants may voluntarily withdraw from the study at any time. Withdrawal of full consent means that the participant does not wish to receive further protocol-required therapy or undergo any study procedures. The sponsor will be notified of all study withdrawals. Participant data and samples collected up to the date of withdrawal of consent will be retained and included in the analyses. Where permitted by local regulations, publicly available data (e.g., death records) may be included after withdrawal of consent.

The investigator will specify reason for discontinuation from the study as follows:

- Participant withdrawal of consent.
- Investigator decision (only for participants who did not receive study drug).
- Loss to follow-up.
- Death.

For participants who are lost to follow-up (defined as 3 documented attempts to contact the participant via all available contact information in a reasonable time period), the investigator should attempt to search publicly available records (where permitted and allowed by local law) to ascertain vital status. For the duration of the study, attempts should also be made to collect information from other sources related to hospitalizations.

8.2. Study Assessments and Procedures

Refer to the schedule of assessments (Table 1) for the timing of the required procedures.

If needed, procedures may occur on separate days, but they must be done within the defined visit window.

8.2.1. Informed Consent

The investigator at each center will ensure that the participant is given full and adequate oral and written information about the nature, purpose, and possible risks and benefits of the study. Participants must also be notified that they are free to discontinue from the study at any time. The participant should be given the opportunity to ask questions and allowed time to consider the information provided.

The participant's signed and dated ICF must be obtained before conducting any study procedures (Section 13.4).

Whenever important new information becomes available that may be relevant to the participant's consent, the written ICF and any other written information provided to participants will be revised by the sponsor or designee and submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) for review. The agreed upon, revised ICF will be provided to each participant in the study for signing and dating. The investigator will explain the changes to the previous version.

8.2.2. Demographics and Medical History

Demographic data, including date of birth, sex, race, and ethnicity, will be collected. Medical history, including a full history of the participant's disease and response to treatment from date of diagnosis, will be obtained. Cardiac and surgical history will also be obtained.

For study entry, all participants must fulfill all inclusion criteria described in Section 4.1, and have none of the exclusion criteria described in Section 4.2.

8.2.3. Physical Exam, Height, and Weight

Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, Day 30, and EOS visits, and the results documented.

Symptom-directed abbreviated physical examination may be performed at all other study visits. Changes noted from the examination performed at screening will be recorded as AEs.

Weight will be obtained according to the schedule of assessments (Table 1).

Height, BMI, and waist/hip ratio will only be obtained at screening and end of study.

8.2.4. Vital Signs

Vital signs will be recorded at every study visit according to the schedule of assessments (Table 1), and will include a single measurement of blood pressure, heart rate, respiratory rate, oxygen saturation by pulse oximetry, and temperature. On Day 1 and during hospitalization, measurements will be performed at the timepoints specified in the schedule of assessments (Table 1). Blood pressure and pulse rate should be measured using the site's equipment.

1. Participant should be seated in a chair with their back supported, feet flat on the floor, and arms bared and supported at heart level.
2. The appropriate cuff size must be used to ensure accurate measurement and used consistently throughout the study.
3. Readings should be done on the same arm at each visit, preferably the non-dominant arm.
4. Measurement should begin after at least 5 minutes of rest.
5. Assessment of pulse rate can be manual or automated. When done manually, the rate should be counted in the brachial/radial artery for at least 30 seconds.

Other procedures should not be performed during blood pressure or heart rate measurements.

Vital sign measurements may be performed by the participants health care provider and reported to the investigator via the template provided by the sponsor.

8.2.5. Liver Imaging

Standard local procedures will be used for image acquisition and analysis. A 3-hour fast is recommended prior to the following imaging procedures. A liver FibroScan or MRE (depending on availability) will be performed at screening and results will be used to exclude patients with liver stiffness consistent with signs of fibrosis (Section 4.2, Exclusion Criterion 2c). A liver

MRI–proton density fat fraction or ultrasound (for assessment of fatty liver/steatosis) will be performed at the screening and EOS visits. Baseline liver fat status and quantitative change in hepatic steatosis will be collected within the case report form (CRF), with clinically significant findings reported as medical history or AEs, as appropriate.

8.2.6. Echocardiogram

A transthoracic echocardiogram (for assessment of left ventricular ejection fraction) will be performed as described in the schedule of assessments (Table 1). Additional echocardiograms may be obtained at the investigator's discretion.

8.2.7. Electrocardiogram

Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion.

8.2.8. Laboratory Tests

Laboratory samples will be collected and analyzed according to the schedule of assessments (Table 1). Unless stated, local laboratories meeting country-specific requirements for clinical testing will be utilized to analyze all tests. Laboratory assessments are listed in Table 4 and Table 5.

Table 4: Local Laboratory Testing

Serum chemistry	ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein
Urinalysis	Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal)
Coagulation	PT, PTT, fibrinogen
Serum or urine pregnancy test	hCG
CBC with differential	Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count
Thyroid function	T3, T4, TSH
Viral serology	HIV-1, HIV-2, HCV antibody and RNA, HBV surface antigen, HBV surface antibody, HBV core antibody

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CBC: complete blood count; eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; HBV: hepatitis B virus; hCG: human chorionic gonadotropin; HCV: hepatitis C virus; HIV: human immunodeficiency virus; MDRD: Modification of Diet in Renal Disease; PT: prothrombin time; PTT: partial thromboplastin time; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone.

Table 5: Central Testing

Genetic testing	*LDLR*, *APOB*, *PCSK9, LPL*, *APOC2*, *APOA5*, *LMF-1*, *GPIHBP1*
Lipid panel	Total cholesterol, TG, HDL-C, non–HDL-C, LDL-C, VLDL-C, Lp(a), ApoB, ApoC-III[1,2]

APOA5: apolipoprotein A5; ApoB or *APOB*: apolipoprotein B; *APOC2*: apolipoprotein C2; ApoC-III: apolipoprotein C-III; *GP1HBP1*: glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1; HDL: high-density lipoprotein; LDL-C: low-density lipoprotein cholesterol; *LDLR*: low-density lipoprotein receptor; *LMF-1*: lipase maturation factor 1; Lp(a): lipoprotein(a); LPL: lipoprotein lipase; *PCSK9*: proprotein convertase subtilisin/kexin type 9; TG: triglycerides; VLDL: very low-density lipoprotein.
[1] Participants on apheresis should have lipid levels sampled within 5 days prior to the procedure (a pre-apheresis sample on the day of apheresis is also adequate).
[2] All lipid panel samples testing to be performed after a minimum of an 8 hour fast.

8.2.9. Pregnancy Testing

All females enrolled in the study must have pregnancy tests performed according to the schedule of assessments (Table 1). Serum pregnancy testing should be performed at screening. A negative serum pregnancy test, performed as per local standards, is required within 72 hours before CTX310 infusion. Urine or serum pregnancy test may be performed as per local standards at the EOS visit.

8.3. Immunogenicity

CTX310 is composed of mRNA encoding SpCas9 and [redacted] sgRNA targeting the gene of interest, encapsulated in an LNP. Blood samples will be collected as described in the schedule of assessments (Table 1) and stored for potential future immunogenicity assessments (anti-drug antibody to LNP and Cas9), if required.

8.4. Pharmacokinetics and Pharmacodynamics Assessments

8.4.1. CTX310 Pharmacokinetic Analysis

PK analysis of [redacted] (LNP), [redacted] (LNP), and Cas9 protein levels will be performed on blood samples collected per the schedule of assessments (Table 1). On Day 1, samples will be collected at prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after the completion of CTX310 infusion. For all other time points, a single sample will be collected.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.2. ANGPTL3

Plasma samples will be obtained to follow the ANGPTL3 concentration, as described in the schedule of assessments (Table 1).

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3. Exploratory Biomarker Research

Exploratory biomarker research may be conducted to identify genomic, metabolic, and/or proteomic biomarkers that may be indicative or predictive of clinical response, resistance, safety, PD activity, and/or the mechanism of action of treatment. In addition, samples collected for protocol-specific endpoints will be used for exploratory research, pending availability of excess sample.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3.1. Whole Blood

Whole blood samples will be obtained and stored at screening.

8.4.3.2. Plasma

Plasma samples for storage will be obtained at screening.

8.4.3.3. Serum

Serum samples will be obtained to follow exploratory biomarkers (e.g., cytokines), as described in the schedule of assessments (Table 1).

9. SAFETY, ADVERSE EVENTS, AND STUDY OVERSIGHT

The investigator will monitor each participant for clinical and laboratory evidence of AEs on a routine basis throughout the study and assess and record details as described in Section 9.1. AEs in response to a query, observed by site personnel, or reported spontaneously by the participant will be recorded. The investigator is responsible for ensuring that any AEs observed by the investigator or reported by the participant are recorded in the participant's medical record in the sponsor's electronic data capture system.

9.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not considered related to the medicinal (investigational) product [Guidelines for Good Clinical Practice (GCP) E6(R2)]. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

The investigator will assess and record information pertaining to the AE, which includes but is not limited to the following: date of onset, event diagnosis (when known) and/or signs and symptoms, duration, severity, seriousness, relationship to the study therapy or procedure, action(s) taken, and outcome.

Additional criteria defining an AE are described below.

The following **are** considered AEs:

- Aggravation of a pre-existing disease or permanent disorder (any clinically significant worsening in the nature, severity, frequency, or duration of a pre-existing condition).
- Events resulting from protocol-mandated procedures (e.g., complications from invasive procedures).

The following **are not** considered AEs:

- Elective or preplanned medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. These should be recorded in the relevant eCRF.

  Note: An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or serious adverse event (SAE).
- Pre-existing diseases or conditions that do not worsen during or after administration of the investigational medicinal product.
- Hospitalization planned for study treatment infusion or observation.

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study participant represents a clinically significant change from the participant's baseline value. Only abnormal laboratory results considered by the investigator

to be clinically significant should be reported as AEs (e.g., an abnormal laboratory finding associated with clinical symptoms, of prolonged duration, or that requires additional monitoring and/or medical intervention). Whenever possible, these should be reported as a clinical diagnosis rather than the abnormal parameter itself (i.e., neutropenia vs neutrophil count decreased). Abnormal laboratory results without clinical significance should not be recorded as AEs.

AEs can occur before, during, or after treatment, and can be either treatment-emergent (AEs that start or worsen on or after CTX310 infusion) or non–treatment-emergent. A non–treatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that occurs after written informed consent has been obtained and before the participant has received CTX310.

9.2. Serious Adverse Event

An AE of any untoward medical consequence must be classified as an SAE if it meets any of the following criteria:

- Results in death.
- Is life-threatening (i.e., an AE that, in the opinion of the investigator, places the participant at immediate risk of death).
- Requires inpatient hospitalization or prolongs an existing hospitalization (hospitalizations for scheduled medical or surgical procedures or to conduct scheduled treatments do not meet these criteria).
- Results in persistent or significant disability or incapacity.
- Results in a congenital anomaly or birth defect in a newborn.
- Other important/significant medical events. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the patient or participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Hospitalization for study treatment infusions or planned hospitalizations following CTX310 infusion are not considered SAEs. Furthermore, hospitalizations for observation or prolongation of hospitalization for observation alone should not be reported as an SAE unless they are associated with a medically significant event that meets other SAE criteria, as assessed by the investigator.

9.3. Adverse Events of Special Interest

An AESI, whether serious or nonserious, is one of scientific and medical concern specific to the sponsor's product or program for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate.

The following events and/or laboratory findings will be designated as AESIs based on the predicted pharmacology, nonclinical safety profile, possible off-target effects, and/or adverse reactions seen in studies of other ANGPTL3 inhibitors (see Investigator's Brochure for details):

- Infusion-related reactions.
- Abnormal coagulation findings, defined as clinically relevant abnormal bleeding, thrombotic, or hemorrhagic events.
- Increase in ALT or AST: ≥3 × ULN or ≥2 × the baseline value (if baseline ALT ≥ULN).
- Allergic reactions/events or localized reactions (collected for 30 days post-infusion).
- New malignancy.

Additional information on the required AESI reporting collection period is detailed in Table 7.

9.4. Adverse Event Severity

AESIs and DLTs will be graded using CTCAE version 5.0. If CTCAE v5.0 grade or protocol-specified criteria are not applicable, AE toxicity should be graded according to Table 6.

Table 6: Adverse Event Severity

Severity Grade	Description
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL.[1]
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.[2]
4	Life-threatening consequences: urgent intervention indicated.
5	Death related to AE.

ADL: activities of daily living; AE: adverse event.
[1] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[2] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

9.5. Adverse Event Causality

The investigator must assess the relationship between each AE and CTX310 and any protocol-mandated study procedure (all assessed individually). The assessment of relationship will be made based on the following definitions:

- **Related**: There is a clear causal relationship between the study treatment or procedure and the AE.
- **Possibly related**: There is some evidence to suggest a causal relationship between the study treatment or procedure and the AE, but alternative potential causes also exist.
- **Not related**: There is no evidence to suggest a causal relationship between the study treatment or procedure and the AE.

Investigators should consider the temporal association between the timing of the event and administration of the treatment or procedure, a plausible biological mechanism, and other

potential causes of the event (e.g., concomitant therapy, underlying disease) when making their assessment of causality.

If an AE is assessed to be not related to any study intervention, an alternative etiology must be provided in the CRF.

If the relationship between the AE and the investigational product is determined to be "possible," a rationale for the assessment must be provided by the reporting investigator.

9.6. Outcome

The outcome of an AE will be classified and reported as follows:

- Fatal.
- Not recovered/not resolved.
- Recovered/resolved.
- Recovered/resolved with sequelae.
- Recovering/resolving.
- Unknown.

9.7. Adverse Event Collection Period

The safety-related information of all participants enrolled in this study will be recorded from the time of ICF signing until end of study; however, there are different reporting requirements for the different time periods in the study. Table 7 describes the AEs that should be reported at each time period of the study.

Table 7: Adverse Event Collection by Study Time Period

Time Period	AE Reporting Requirements
Informed consent to 30 days after CTX310 infusion	All AEs
30 days after CTX310 infusion through Month 12 visit	• Nonserious AEs related to study procedure[1] or CTX310 • SAEs • AESIs

AE: adverse event; AESI: adverse event of special interest; IV: intravenous; SAE: serious adverse event.
[1] AEs related to study procedures include events related to, e.g., laboratory procedures, radiography, or IV treatment infusions, and may occur at any time starting at screening and continuing through the duration of the study.

If a participant does not receive CTX310 therapy after enrollment, the AE reporting period ends 30 days after last study-related treatment or procedure (e.g., pretreatment regimen, biopsy, imaging).

9.8. Adverse Event Reporting

All AEs will be recorded in the appropriate section of the eCRF. Participants withdrawn from the study because of AEs will be followed by the investigator until the outcome is determined. When appropriate, additional written reports and documentation will be provided.

AE reporting should occur as per the study period designated in Table 7. If a reportable SAE or AESI occurs, the SAE/AESI form provided to investigators should be completed and submitted to the sponsor or its designee immediately (i.e., no more than 24 hours after the investigator becomes aware of the event) by scanning and emailing the paper report to:

globalpv@crisprtx.com (for notifications or questions)

In particular, if the SAE is fatal or life-threatening, the report form must be submitted immediately, irrespective of the extent of available AE information. The timeframe also applies to additional, new information (follow-up) on previously reported SAE/AESI reports.

In the rare event that the investigator does not become aware of the occurrence of a reportable SAE/AESI immediately (e.g., a study participant initially seeks treatment elsewhere), the investigator is to report the event within 24 hours of learning of the event and document the date and time of awareness of the event.

In addition, an investigator may be requested to obtain specific additional follow-up information in an expedited fashion (e.g., autopsy finding). The information collected for SAEs/AESIs is more detailed than that captured on the AE CRF. In general, this will include a description of the event in sufficient detail, as well as concomitant medications and any relevant medical details to allow for a complete medical assessment of the case and causality assessment by the investigator and the sponsor. Information on the other possible causes of the event, such as concomitant medications and illnesses must be provided.

The investigator must complete, sign, and date the SAE/AESI form, verify the accuracy of the information recorded on the SAE/AESI form with the corresponding source documents, and send a copy by email or fax to the sponsor or designee. Subsequently, all SAEs/AESIs will be reported to the health authorities per local reporting guidelines.

It is the principal investigator's responsibility to notify the IRB or EC of all SAEs that occur at his or her site as per local policies. Investigators will also be notified of all unexpected, serious, drug-related events that occur during the clinical study. Each site is responsible for notifying its IRB or EC of these additional SAEs.

9.9. Pregnancy

Certain information, although not considered an AE or SAE, must be recorded, reported, and followed as indicated for an SAE (see Section 9.8), including pregnancies.

Pregnancies (both those of female participants and female partners of male participants) must be reported to the sponsor or designee within 24 hours of the investigator's knowledge using the Investigational Product Pregnancy Report. All pregnancies will be followed through to outcome and the outcome must be reported to the sponsor or designee using the pregnancy outcome section of Investigational Product Pregnancy Report.

Pregnancies themselves are not considered AEs or SAEs. However, any AEs or SAEs occurring during pregnancy are to be reported following AE and SAE reporting guidelines.

9.10. Reporting Deaths

Regardless of relationship to the investigational product, all deaths on study should be recorded in the relevant eCRF. Additionally, all SAEs with outcome of death, regardless of relationship to investigational product, should be reported to the sponsor as SAEs to Pharmacovigilance on the provided SAE/AESI form.

9.11. Study Oversight

9.11.1. Safety Review Committee

During dose escalation, an SRC consisting of investigators and sponsor representatives will review all available safety data and make decisions regarding dose escalation or de-escalation. During dose escalation, the SRC may also propose revisions to the DLT definitions and dosing schema and will continue to meet regularly to discuss toxicity management algorithms and review individual participant cases. Following discussion with the SRC, the sponsor may consult with the independent DSMB regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema.

During follow-up, the SRC may continue to meet on an ad hoc basis to discuss 1 or more of the following: (1) single-participant case studies, (2) aggregate safety and/or biomarker data, (3) toxicity management algorithms, and (4) review and discuss the possibility of expanding the study to include other study populations.

9.11.2. Independent Data Safety Monitoring Board

An independent DSMB comprised of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise to monitor the study will be established during dose escalation. Throughout the study the DSMB will review safety and efficacy data from dose escalation and endorse the RP2D. The DSMB will review safety and efficacy data pertaining to stopping rules provided by the sponsor, as detailed in the DSMB charter. The DSMB may recommend that the sponsor amend the protocol, stop enrollment, or discontinue the study at any time if concerns about safety of the participants are encountered. The roles and responsibilities of the DSMB will be further described in the DSMB charter.

10. STOPPING RULES AND STUDY TERMINATION

10.1. Stopping Rules for the Study

The occurrence of laboratory results meeting any of the following criteria will result in immediate suspension of accrual to the study for safety reasons pending review of the data by the sponsor, the SRC, and the DSMB. Laboratory values that are not confirmed due failure to retest or missing laboratory values will be presumed confirmed.

Participants who have already received a dose of study drug will continue with follow-up as outlined in the protocol. No further dosing of participants will occur until an assessment of the data is completed by the sponsor, the SRC, and the DSMB. Notification of the suspension of accrual will be provided to the health authorities and ECs. If deemed appropriate based on the sponsor, the SRC, and the DSMB review, a rationale with data supporting continued dosing without a change to the protocol will be submitted to the health authorities and ECs for review and approval. If a modification is required, a protocol amendment will be submitted.

Study accrual will be suspended as described above in this section if any of the following criteria are met:

1. ALT or AST >8 × ULN, which is confirmed by repeat testing.
2. ALT or AST >5 × ULN, which is confirmed and persists for >4 weeks.
3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5.
4. Thrombosis, hemorrhage, and/or laboratory parameters consistent with disseminated intravascular coagulation or bleeding that are likely related to study drug.
5. Fatal or life-threatening AE that is due to study drug.
6. Any other AE that poses an immediate hazard to study participants, in the opinion of the sponsor in consultation with the investigators and DSMB.

10.2. Stopping Rules for Individual Participants

Stopping rules for individual participants are as follows:

- Any medical condition that, in the opinion of the investigator or sponsor, would put the participant at risk during continuing study-related treatments or follow-up.
- If a participant is found to have not met all the eligibility criteria or has a major protocol deviation before the start of CTX310 infusion and in the opinion of the investigator.
- Would put the participant at risk for continuing study-related procedures or follow-up.

10.3. Study Termination

This study may be discontinued at any time due to safety concerns, failure to meet expected enrollment goals, administrative reasons, or at the discretion of the sponsor. In the event of study termination, the sponsor will immediately inform all appropriate parties, including principal investigators, ECs, IRBs, and competent authorities. In the event this study is terminated early, participants who have received CTX310 will be asked to participate in a separate long-term follow-up study for up to 15 years post-infusion.

11. STATISTICAL ANALYSES

11.1. Study Objectives and Hypotheses

The primary objective is to evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or LDL-C and/or ApoB, and to determine the RP2D.

The secondary objectives are to assess the preliminary efficacy, PK, and PD of CTX310. No formal hypothesis testing will be performed.

11.2. Study Endpoints

11.2.1. Primary Endpoints

- Incidence of AEs, including treatment-emergent adverse events (TEAEs), AESIs, DLTs; clinically significant laboratory abnormalities; and clinically significant abnormal vital signs.

11.2.2. Secondary Endpoints

11.2.2.1. Secondary Efficacy Endpoints

- Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline.

11.2.2.2. Secondary Pharmacokinetics/Pharmacodynamics Endpoints

- Plasma levels of LNP ([redacted] and [redacted]).
- Plasma level of Cas9 protein.
- Percentage change in ANGPTL3 concentration over time compared to baseline.

11.2.3. Exploratory Endpoints

- Percentage change in FFA levels over time compared to baseline.
- Change in fatty liver disease.
- Immunogenicity of CTX310 (samples will be stored and evaluated for anti-drug antibody to LNP and Cas9, if required).

11.3. Analysis Sets

The following analysis sets will be evaluated and used for presentation of the data.

- The enrolled set includes all participants who sign the informed consent and meet the inclusion/exclusion criteria.
- The safety analysis set is a subset of the enrolled set that includes participants who receive the CTX310 infusion. Analyses of the safety assessments will be based on the

safety analysis set. Participants in the safety analysis set will be classified by received CTX310 dose level.

- The full analysis set (FAS) is a subset of the safety analysis set that includes participants who receive CTX310 infusion and have at least 1 post-baseline lipid assessment or discontinue earlier. The efficacy analyses will be performed based on the FAS. Participants in the FAS will be classified by received CTX310 dose level.

11.4. Sample Size

The sample size of the study will be approximately 24 participants.

11.5. Interim Analysis

No formal efficacy interim analysis is planned.

The DSMB will review safety and efficacy data as needed during the study to monitor stopping rules and to provide recommendations on enrollment or protocol amendment.

11.6. Planned Method of Analyses

The primary analysis will occur after all participants have completed 26 weeks of follow-up after CTX310 infusion or discontinued earlier. A final analysis will occur when all participants complete or withdraw from the study. Tabulations will be produced for appropriate disposition, demographic, baseline, efficacy, and safety parameters. By-participant listings will be provided for all data, unless otherwise specified.

11.6.1. Efficacy Analysis

The FAS will be used as the analysis set for efficacy. The efficacy endpoints of percentage change in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each dose level. Categorical summaries at selected time points, including 26 and 52 weeks, based on appropriate cutoff may be provided.

11.6.2. Safety Analysis

Safety analysis will be conducted on the safety analysis set. Summaries of AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each dose level of CTX310 and overall.

Summaries of AEs will focus on TEAEs, defined as AEs that start or worsen on or after CTX310 infusion. AEs will be graded according to CTCAE v5.0. The incidence of TEAEs will be summarized by system organ class and preferred term, grade, and relation to CTX310. Key subsets of TEAEs, including DLTs, AESIs, grade ≥3 AEs, related AEs, and SAEs, will be summarized separately.

Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of

clinically significant laboratory abnormalities and clinically significant abnormal vital signs will be summarized.

The incidence of other clinically significant safety measure abnormalities (e.g., ECG) will also be summarized, if applicable.

11.6.3. Pharmacokinetic and Pharmacodynamic Analyses

Plasma levels of LNPs ([redacted] and [redacted]) and Cas9 protein over time will be summarized using descriptive statistics. Exploratory analysis based on an applicable PK model may be performed.

Percentage change in ANGPTL3 concentration over time compared to baseline will be summarized using descriptive statistics.

11.6.4. Biomarker Analyses

Additional exploratory biomarkers, including FFA levels, fatty liver disease marker(s), and immunogenicity marker(s), if data are available, will be summarized using descriptive statistics.

11.6.5. Patient-reported Outcome Analyses

Not applicable.

12. DATA MANAGEMENT

12.1. Data Recording and eCRF Processing

The investigator is required to maintain adequate and accurate medical records designed to record all observations and data pertinent to the study for each participant. Study data for each consented participant will be entered into a CRF by site personnel using a secure, validated (Part 11 of Title 21 of the Code of Federal Regulations–compliant), web-based electronic data capture application. Instances of missing, discrepant, or uninterpretable data will be queried by the sponsor or designee for resolution. Any changes to study data will be made to the eCRF and documented in an audit trail maintained within the clinical database. CRFs must be reviewed and electronically signed and dated by the investigator.

An audit may be performed at any time during or after completion of the clinical study by sponsor personnel or their designee. All study-related documentation must be made available to the designated auditor.

13. ADMINISTRATIVE

13.1. Institutional Review Board/Ethics Committee

This protocol and the proposed ICF must be reviewed and approved by the appropriate IRB/EC prior to the start of the study. During the study, the investigator shall make timely and accurate reports to the IRB/EC on the progress of the study at intervals not exceeding 1 year, as well as satisfying any other local IRB/EC regulations regarding reporting. Copies of all reports to and correspondence with and from the IRB/EC must be provided to the sponsor or its designee.

Any significant changes or revisions in the study protocol or any changes that may alter participant risk must be approved in writing by the IRB/EC prior to implementation. A protocol change intended to eliminate an apparent imminent hazard may be implemented immediately provided that the sponsor is promptly notified, and an amendment is subsequently provided by the sponsor and approved by the IRB/EC.

It is the investigator's obligation to maintain an IRB/EC correspondence file, and to make this available for review by sponsor representatives or their designee as part of the study monitoring process.

13.2. Study Conduct

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Guidelines for GCP and applicable regulatory requirements.

13.3. Participant Privacy

To maintain participant confidentiality and to comply with applicable data protection and privacy laws and regulations, all data provided to the sponsor or designee, study reports, and communications relating to the study will identify participants by assigned participant numbers, and access to participant names linked to such numbers shall be limited to the site and the study investigator and shall not be disclosed to the sponsor or designee. As required by applicable laws and regulations in the countries in which the study is being conducted, the investigator will allow the sponsor and/or its representatives access to all pertinent medical records to allow for the verification of data gathered and the review of the data collection process. The regulatory authorities in other jurisdictions, including the IRB/EC, may also request access to all study records, including source documentation, for inspection.

13.4. Written Informed Consent

The investigator will be responsible for obtaining written informed consent from potential participants prior to any study-specific screening and entry into the study. The source documents for each participant shall document that the informed consent was obtained prior to participation in the study.

The investigator at each center will ensure that the participant is given full and adequate oral and written information about the nature, purpose, and possible risk and benefit of the study. Participants must also be notified that they are free to discontinue from the study at any time.

The participant should be given the opportunity to ask questions and allowed time to consider the information provided. The investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the participant. Whenever important new information becomes available that may be relevant to the participant's consent, the written ICF and any other written information provided to participants will be revised by the sponsor or designee and be submitted to the IRB/EC for review and favorable opinion. The agreed upon, revised information will be provided to each participant in the study for signing and dating. The investigator will explain the changes to the previous version.

13.5. Delegation of Investigator Responsibilities

The investigator will ensure that all persons involved in the conduct of the study are informed about the protocol, protocol amendments, study procedures, and study-related duties.

13.6. Study Files

Documentation concerning investigators' credentials and experience, and IRB approval of protocol and ICF, and other documentation are required prior to shipment of the investigational product to the study site. Copies of these documents as well as supplemental information, such as the Investigator's Brochure, will be kept onsite in an investigator study file binder. This file also will contain investigational product accountability (receipt/dispensing) records, sponsor/investigator correspondence, IRB correspondence, changes to the protocol, information regarding monitoring activities, participant exclusion records, and biological sample records.

13.7. Retention of Study Documents

All study documents, including records of investigational product receipt and disposition, copies of eCRFs, as well as supporting documentation and administrative records, must be retained by the investigator for a minimum of 2 years following notification that the appropriate regulatory authority has approved the product for the indication under study, notification that the entire clinical investigation will not be used in support of a marketing application, or notification that the marketing application was not approved. No study documents will be destroyed or moved to a new location without prior written approval from the sponsor. If the investigator relocates, retires, withdraws from the clinical study for any reason, or dies, all records required to be maintained for the study should be transferred to an agreed upon designee, such as the study monitor, another investigator, or the institution where the study was conducted. The sponsor should be notified in writing at least 30 days prior to the disposal of any study records related to this protocol.

13.8. Protocol Compliance

No modifications to the protocol will be made without the approval of both the investigator and the sponsor. Changes that significantly affect the safety of the participants, the scope of the investigation, or the scientific quality of the study (e.g., efficacy assessments) will require IRB/EC notification before implementation, except where the modification is necessary to eliminate an apparent immediate hazard to human participants. The sponsor will submit all protocol modifications to the required regulatory authorities.

Emergency departures from the protocol that eliminate an apparent immediate hazard to a particular participant and that are deemed crucial for the safety and well-being of that participant may be instituted for that participant only. The investigator or other attending physician also will contact the sponsor as soon as possible in the case of such a departure. These departures do not require preapproval by the IRB; however, the IRB and sponsor must be notified in writing as soon as possible after the departure has been made. In addition, the investigator will document the reasons for protocol deviation and the ensuing events.

13.9. Monitoring Functions and Responsibility

Before an investigational site can enter a participant into the study, a sponsor representative will evaluate the investigational study site to:

- Determine the adequacy of the facilities.
- Discuss with the investigator(s) and other personnel their responsibilities regarding protocol adherence, and the responsibilities of the sponsor or its representatives. This will be documented in a Clinical Study Agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or representative will have regular contacts with the investigational site to:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, and that investigational product accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the CRFs with the participant's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each participant (e.g., clinic charts).
- Record and report any protocol deviations not previously sent to the sponsor.
- Confirm AEs and SAEs have been properly documented on CRFs, that any SAEs and AESIs have been forwarded to the sponsor, and SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

13.10. Quality Control and Quality Assurance

Authorized representatives of the sponsor, a regulatory authority, an EC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded,

analyzed, and accurately reported per the protocol, ICH/GCP guidelines, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

13.11. Disclosure of Data

All information obtained during the conduct of this study will be regarded as confidential. Disclosures (i.e., any release of information to any third party not noted herein) of any information not previously known to be public and/or results of the investigation for publication or by capsules or poster presentation shall not be made earlier than 30 days after submission of the proposed material to the sponsor for inspection, unless the sponsor consents to earlier disclosure. The investigator will take appropriate cognizance of the sponsor's suggestions before disclosure for publication or presentation consistent with protection of the sponsor's right to its confidential data.

13.12. Confidentiality and Publication

All scientific, commercial, and technical information disclosed by the sponsor in this protocol or elsewhere will be considered the confidential and proprietary property of the sponsor. The investigator will hold such information in confidence and shall not disclose the information to any third party except to such of the investigator's employees and staff as have been made aware that the information is confidential and who are bound to treat it as such and to whom disclosure is necessary to evaluate that information. The investigator will not use such information for any purpose other than determining mutual interest in performing the study and, if the parties decide to proceed with the study, for the purpose of conducting the study.

The investigator understands that the information developed from this clinical study will be used by the sponsor in connection with the development of the investigational product and other drugs and diagnostics, and therefore may be disclosed as required to other clinical investigators, business partners and associates, the FDA, and other government agencies. The investigator also understands that to allow for the use of the information derived from the clinical study, the investigator has the obligation to provide the sponsor with complete test results and all data developed in the study.

Authorship of publications will be determined based on the Recommendations for Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, which states that authorship should be based on the following 4 criteria:

1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;
2. Drafting of the article or revising it critically for important intellectual content;
3. Final approval of the version to be published; and
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

An individual must meet all criteria to be an author on any publication containing data from this study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between the sponsor and the investigator and/or the investigator's institution.

13.13. Clinical Study Report

After completion of the study, a clinical study report, written by the sponsor or designee in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

14. APPENDIX: DEFINITIONS AND SUPPORTING OPERATIONAL DETAILS

14.1. Lean Body Weight Calculation

Estimated lean body weight (eLBW) will be estimated using the equation developed and validated by Janmahasatian et al, (Janmahasatian et al., 2005).

Females: LBW[kg] = (9270 × Total_body_weight[kg]) / (8780 + (244 × BMI[kg/m^2]))

Males: LBW[kg] = (9270 × Total_body_weight[kg]) / (6680 + (216 × BMI[kg/m^2]))

Where BMI[kg/m^2] = Total_body_weight[kg] / Height[m]^2

14.2. Contraception Requirements

Females of childbearing potential are excluded from the study.

Female Participants of Nonchildbearing Potential

Female participants of nonchildbearing potential will not be required to use contraception. To be considered of nonchildbearing potential, female participants must meet at least 1 of the following criteria:

- Postmenopausal: At least 12 consecutive months of amenorrhea in women with a uterus without an alternative medical cause; **or**
- Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).

Note: All other female participants (including participants with tubal ligations and participants who do not have a documented hysterectomy) will be considered of childbearing potential.

Acceptable methods of contraception for male participants and their partners are listed below. If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.

Male Participants

Acceptable contraceptive methods must be used from study informed consent through 12 months after CTX310 infusion and include the following:

- Nonsterile male participants who are or may become sexually active with female partners of childbearing potential must agree to use an acceptable, effective method of contraception from study informed consent through 12 months after CTX310 infusion.
- If the male is infertile (e.g., bilateral orchiectomy). Infertility may be documented through examination of a semen specimen or by demonstration of the absence of the vas deferens by ultrasound.
- True abstinence for the participant, when this is in line with the preferred and usual lifestyle of the participant. Periodic abstinence (e.g., calendar, ovulation,

symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.

- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise, condom and spermicide as separate products). Local regulations may require use of an additional acceptable method of contraception.
- Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of mobilization, and 1 barrier method of contraception.

Acceptable contraceptive methods for female partners of male participants:

- Bilateral tubal ligation performed at least 6 months previously.
- Continuous use of an intrauterine device for at least 90 days before consent.
- Hormonal contraceptives, if successfully used for at least 60 days before consent.

Additional notes:

- Female condom cannot be used with male condom (as a double method of contraception) due to risk of tearing.
- The use of birth control methods does not apply if the female partner has had a bilateral oophorectomy, hysterectomy, or is postmenopausal.
- Male participants who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.
- If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.
- Male participants must not donate sperm throughout the study, and for 12 months following CTX310 infusion.
- Unique situations that may not fall within the above specifications may be discussed with the sponsor medical monitor or designee on an individual basis.

14.3. Country/Region-Specific Differences

Not applicable for this version of the protocol.

14.4. Prior Protocol Amendments and Protocol History

14.4.1. Summary of Changes to the Current Protocol Version

The Protocol Amendment Summary of Changes for the current amendment is located directly before the Table of Contents. The current protocol is Version 2.0, Amendment 1. The protocol history is tabulated in Section 14.4.2.

14.4.2. Protocol History

Document/Version	Date	Global/Country/Site Specific
Original Protocol/Version 1.0	27 January 2023	Global
Protocol Amendment 1/Version 2.0	05 May 2023	Global

15. APPENDIX: GLOSSARY OF TERMS

List of Abbreviations

Abbreviation	Term
ACC	American College of Cardiology
AE	adverse event
AESI	adverse event of special interest
AHA	American Heart Association
ALT	alanine aminotransferase
ANGPTL3	angiopoietin-like 3
AP	alkaline phosphatase
ApoB	apolipoprotein B
ASCVD	atherosclerotic cardiovascular disease
AST	aspartate aminotransferase
BMI	body mass index
Cas9	CRISPR-associated protein 9
CCS	Canadian Cardiovascular Society
CNS	central nervous system
CRF	case report form
CRISPR	clustered regularly interspaced short palindromic repeats
crRNA	crisprRNA
CTCAE	Common Terminology Criteria for Adverse Events
CVD	cardiovascular disease
DL	Dose Level
DLT	dose-limiting toxicity
DP	drug product
DS	drug substance
DSB	double-stranded break
DSMB	Dug Safety Monitoring Board
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form
EL	endothelial lipase
eLBW	Estimated lean body weight
EOS	end of study
FAS	full analysis set

Abbreviation	Term
FCS	familial chylomicronemia syndrome
FDA	Food and Drug Administration
FFA	free fatty acids
FIH	first-in-human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GOF	gain-of-function
GP1HBP1	glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1
HDL-C	high-density lipoprotein cholesterol
HDR	homology directed repair
HeFH	heterozygous familial hypercholesterolemia
HIV	human immunodeficiency virus
HoFH	homozygous familial hypercholesterolemia
HTG	hypertriglyceridemia
ICF	informed consent form
ICH	International Conference on Harmonisation
IDL	intermediate-density lipoprotein
IgM	immunoglobulin M
indel	insertion/deletion mutation
INR	international normalized ratio
IRB	Institutional Review Board
IRR	infusion-related reaction
IV	intravenous
kPa	kilopascals
LDL-C	low-density lipoprotein cholesterol
LDLR	low-density lipoprotein receptor
LFT	liver function test
LNP	lipid nanoparticle
LOF	loss-of-function
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
MCS	multifactorial chylomicronemia syndrome
MDRD	Modification of Diet in Renal Disease
mIU	milli international units
MRE	magnetic resonance elastography

Abbreviation	Term
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
NAT	nucleic acid testing
NHEJ	nonhomologous end joining
NHP	non-human primate
NOAEL	no-observed-adverse-effect level
OBD	optimum biological dose
PAM	protospacer adjacent motif
PCSK9	proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamic
PK	pharmacokinetic
RNA	ribonucleic acid
RP2D	recommended Phase 2 dose
SAE	serious adverse event
sgRNA	single-guide ribonucleic acid
SpCas9	*Streptococcus pyogenes* CRISPR-associated protein 9
SRC	Safety Review Committee
TEAE	treatment-emergent adverse event
TG	triglyceride(s)
tracrRNA	trans-activated crisprRNA
ULN	upper limit of normal
VLDL	very low-density lipoprotein

16. APPENDIX: REFERENCES

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CLINICAL STUDY PROTOCOL

CRSP-CVD-400

A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias

Study Drug: CTX310
Study Phase: 1
Date of Original Protocol: 27 January 2023

Date of Protocol Amendment: 18 April 2025, Amendment 5
Version: 6.0

Sponsor Emergency Contact	[redacted]	[redacted]

CRISPR Therapeutics AG
Baarerstrasse 14
CH 6300 Zug, Switzerland

SIGNATURE PAGES

PROTOCOL APPROVAL SIGNATURE PAGE

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	18 April 2025
Version	6.0
Amendment	5

Reviewed and approved by:

{see electronic signature page at the end of the document.}

[redacted]
Medical Monitor
CRISPR Therapeutics AG

Date

PROTOCOL ACCEPTANCE FORM

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	18 April 2025
Version	6.0
Amendment	5

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice, and all applicable regulatory requirements.

Investigator's Signature _____ Date _____

Name (printed) _____

AMENDMENT DETAILS

History of Amendments

Four prior global amendments have occurred, and the summary of all amendments is provided in Section 14.4.2 through Section 14.4.5. The protocol history is documented in Section 14.4.6.

Summary of Key Changes to the Current Protocol Version 6.0, Amendment 5 (Global)

The previous version of this protocol (Version 5.0, 03 June 2024) was amended to create the current version (Version 6.0, 18 April 2025).

This is a substantial amendment to the protocol. The primary reason for this amendment is:

- Replacement of the confirmatory cohort from Phase 1a with Phase 1b disease-specific cohort expansion using a flat dose within a recommended range approved by the Safety Review Committee (SRC) following evaluation of the totality of pharmacokinetic (PK)/pharmacodynamic (PD), key lipid and safety data from dose escalation based on estimated lean body weight in mg/kg of CTX310.
- Addition of 4 disease specific treatment cohorts in a Phase 1b cohort expansion: severe hypertriglyceridemia, homozygous familial hypercholesterolemia, heterozygous familial hypercholesterolemia and refractory mixed hyperlipidemias.
- Addition of a disease-specific exploratory objective/endpoint.
- Addition of disease specific inclusion/exclusion criteria.
- Update of pre-infusion prophylaxis regimen.

All key changes made in the current version of the protocol are tabulated as Summary of Changes to the Current Protocol with the rationale and section impacted in Section 14.4.1.

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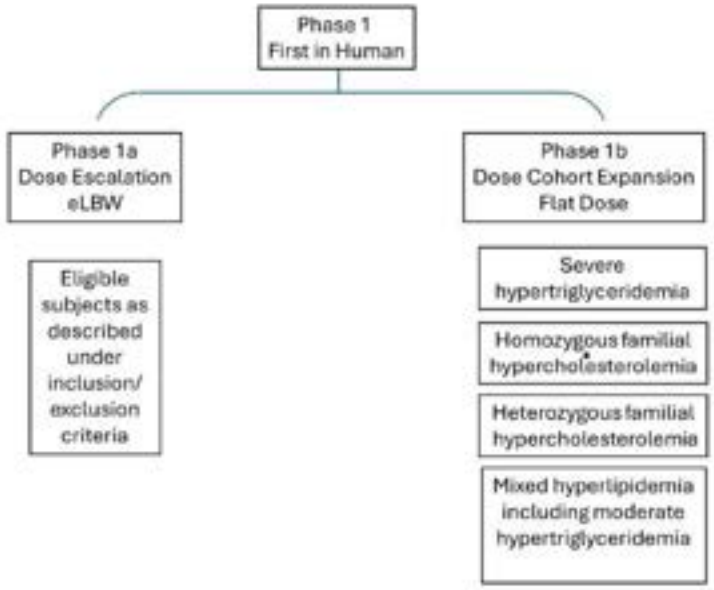
1. PROTOCOL SUMMARY

1.1. Protocol Synopsis

Sponsor: CRISPR Therapeutics AG	**Protocol Number**: CRSP-CVD-400
Name of Investigational Product: CTX310	**Phase of Development**: 1
Protocol Title: A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR-Guide RNA-Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias	
Number of Subjects: Phase 1: Approximately 69 Phase 1a Dose Escalation: Approximately 21 Phase 1b Disease-specific Cohort Expansion: Approximately 48	
Investigators: Multicenter	**Study Type:** Interventional
Investigational Product Description CTX310 is a lipid nanoparticle (LNP) formulation of clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) components for in vivo editing of the target gene angiopoietin-like 3 (*ANGPTL3*). The investigational drug product consists of a capped and polyadenylated spacer Cas9 messenger ribonucleic acid containing N1-methylpseudouridine and a 100 nucleotide–long single-guide ribonucleic acid targeting the gene of interest. CTX310 is designed to utilize CRISPR-Cas9 to disrupt exon 1 of human *ANGPTL3* in the liver, leading to a decrease of ANGPTL3 protein levels.	
Mode of Administration: Subjects will receive CTX310 via intravenous (IV) infusion.	
Study Population The study population will consist of subjects 18 to 75 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride(s) (TG) and/or non–high-density lipoprotein cholesterol (non-HDL-C), including low-density lipoprotein cholesterol (LDL-C), and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTDs) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population).	

Figure S1 Study Design



The Phase 1a dose escalation part of this study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:

- Multifactorial chylomicronemia syndrome (MCS).
- Homozygous familial hypercholesterolemia (HoFH).
- Heterozygous familial hypercholesterolemia (HeFH)
- Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).
- Other HTG/hypercholesterolemia syndromes of undetermined etiologies.

The Phase 1a dose escalation will include eligible subjects, regardless of underlying dyslipidemia subtype. The majority of subjects enrolled in Phase 1a are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia and HTG. Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk.

The Phase 1b disease-specific cohort expansion will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD that encompass HTG and/or familial hypercholesterolemia syndromes in the following disease-specific cohorts:

- Severe HTG (>500 mg/dL triglycerides)
- HoFH
- HeFH
- Mixed hyperlipidemias (including moderate HTG)

Subjects will be asked to continue to take their baseline lipid-lowering medications in the same doses through the study period until a significant beneficial effect (i.e., achievement of target lipid goals) of CTX310 is observed.

Duration of Participation: All subjects will be monitored for safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) effects for 12 months post-infusion in the study. All subjects will be asked to consent to participate in a separate long-term follow-up (LTFU) study for up to 15 years post-infusion.

Objectives and Endpoints

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of DLTs and frequency of AEs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non-HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To further characterize the safety of CTX310	• Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline

AE: adverse event; AESI: adverse event of special interest; [redacted]

ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; DLT: dose-limiting toxicity; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; PD: pharmacodynamic; PK: pharmacokinetic; TEAE: treatment-emergent adverse event; TG: triglyceride(s).

Study Design:

This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll approximately 45 subjects 18 to 75 years (inclusive) of age with dyslipidemias and increased levels of TG (>150 mg/dL [1.7 mmol/L]; >500 mg/dL [5.65 mmol/L] for severe hypertriglyceridemia) and/or LDL-C (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for ASCVD) and/or non–HDL-C (>160 mg/dL [4.2 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L]) that are refractory to indicated and available treatments.

Phase 1a - Dose Escalation

Three to 6 subjects will be enrolled in each of the dose levels (DLs): 0.1, 0.3, 0.6, 0.8, 1.0, and 1.2 mg/kg estimated lean body weight (eLBW) of total ribonucleic acid (RNA) in the LNP formulation. Each subject will receive a single IV dose of CTX310 and will be hospitalized for a minimum of 24 hours after CTX310 infusion (or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment) and will be closely monitored post-infusion for adverse events (AEs) defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All subjects will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Details of the toxicity management guidelines are provided in the protocol.

When the DLT evaluation period ends for the last subject enrolled at each escalation DL, the Safety Review Committee (SRC) will review pharmacokinetic (PK)/pharmacodynamic (PD) and safety data and will be responsible for making decisions regarding dose escalation or de-escalation.

Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D). The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.

Phase 1b – Disease-specific Cohort Expansion

Following completion of eLBW-based Phase 1a dose escalation, the SRC will review the totality of safety and clinical activity data to delineate an FD for Phase 1b or anticipated RP2D. The FD will be applied to the Phase1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease-specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. An additional FD may be evaluated in each expansion cohort if the safety or activity profile of the first FD is inadequate and will not exceed the equivalent highest dose evaluated during Phase 1a. Administration of CTX310 in the Phase 1b disease-specific cohort expansion part of the study will occur in the same manner as in Phase 1a. Phase 1b will confirm the RP2D.

The SRC will be responsible for endorsing the RP2D.

After CTX310 infusion, subjects will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on *ANGPTL3* expression and lipid profile. All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months and those who complete the study, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion.

At each DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the SRC before proceeding to the next DL.

Each subject will undergo the following stages:

1. Screening: up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All subjects will be monitored for safety, tolerability, PK, and PD effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: Roll over to a separate LTFU study for up to 15 years post-infusion.

Study Oversight

Safety Review Committee

An SRC consisting of investigators and sponsor representatives will review all available safety data when the DLT observation period ends for the last subject enrolled in each Phase 1a DL and will be responsible for making decisions regarding dose escalation or de-escalation. Throughout dose escalation, for cases in which a dose had been cleared in a DL and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to enroll an additional number of subjects for a total of up to 6 subjects at the current DL to gather additional safety data. The SRC will continue to meet regularly during the dose escalation phase to discuss toxicity management algorithms and to review individual subject cases. Following discussion with the SRC, the sponsor may consult with the independent Data Safety Monitoring Board (DSMB) regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema.

Following completion of dose escalation and analysis of PK/PD, key lipid, and safety data in Phase 1a, the SRC will evaluate the totality of clinical data to approve a FD for evaluation in Phase 1b expansion cohorts.

The SRC may be consulted on other aspects of the study conduct, as applicable.

Independent Data Safety Monitoring Board

An independent DSMB consisting of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise will be formed at the start of the study, and roles and responsibilities will be described in the DSMB charter. Throughout the study the DSMB will review safety from dose escalation and disease-specific cohort expansions. The sponsor or designee will be responsible for alerting the DSMB regarding any suspected, unexpected, serious adverse reaction related to CTX310.

Proposed Starting Dose and Dose Escalation

The CTX310 doses in Table S1 are proposed for evaluation in the 6 planned dose escalation levels in the study, with a minimum of 3 and a maximum of 6 evaluable subjects per DL. The first-in-human (FIH) starting dose is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the non-human primate (NHP) Good Laboratory Practice (GLP) safety and toxicology study (refer to CTX310 Investigator's Brochure).

The initial starting dose of 0.1 mg/kg eLBW of CTX310 refers to the total RNA dose that is based on a NOAEL of 1 mg/kg in the GLP safety and toxicity study. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg lean body weight (LBW). The emerging clinical data on systemically infused LNP-associated therapeutics demonstrates a relatively safe profile. A 3-fold safety factor is proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies. With an anticipated 3- to 2-fold increment in DLs, dose escalation is expected to proceed from 0.1 to 1.2 mg/kg eLBW. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data (Section 5.5).

Table S1: Phase 1a Dose Escalation of CTX310

Dose Level*	Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6
4	0.8 [2]
5	1.0 [3]
6	1.2 [4]

DL: Dose Level; eLBW: estimated lean body weight; RNA: ribonucleic acid; SRC: Safety Review Committee.

* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose.

[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW.

[2] Following review of clinical data by the sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.

[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.

[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.

Dosing Within a Dose Level in Phase 1a Dose Escalation

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 subjects will be treated at each DL depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in LFTs observed after dosing with CTX310 resolved within 14 days, the dosing between each subject within a DL will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs) have returned to $<2 \times$ baseline or to normal levels, whichever is later. If the safety evaluation of a subject is acceptable, the next subject in the DL may be dosed.

Dose escalation may proceed when all subjects in the preceding DL have completed dosing, the last subject has completed ≥30-day safety evaluation, and the cumulative safety data of all treated subjects at that DL demonstrate an acceptable safety profile, as determined by the SRC.

Rules for Dose-limiting Toxicity Assessment During Phase 1a Dose Escalation

Subjects must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable subject (i.e., a subject that has been administered CTX310 and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable subjects are required per DL during Phase 1a.

An adequate interval will be applied between current lipid-lowering treatments a subject is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities.

Note: Any subject who experiences a DLT will be considered evaluable. Data for all subjects who receive CTX310 will be part of the safety analysis set.

Dose escalation will be performed according to the following rules:

- If 0 of 3 subjects experience a DLT, escalate to the next DL.
- If 1 of 3 subjects experiences a DLT, expand the current DL to 6 subjects.

- If 1 of 6 subjects experiences a DLT, escalate to the next DL.
- If ≥2 of 6 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL.

- If ≥2 of 3 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 or any optional de-escalation DL as specified in the table above, de-escalate to previous DL or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- No dose escalation beyond highest dose planned or listed for the study (Table S1). Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones in the DL table above, may be added during the course of dose escalation to support the identification of a safe and effective dose.

Phase 1b – Disease-specific Cohort Expansion

Following completion of eLBW-based dose escalation (Table S1), analysis of PK/PD, key lipid and safety data, and approval by the SRC, a safe and efficacious FD will be evaluated in 4 disease-specific cohort expansion cohorts. Each disease-specific cohort may enroll up to 12 subjects.

Following endorsement from the SRC, the sponsor will declare the RP2D based on the totality of clinical data for each disease-specific cohort for future studies. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 during dose escalation may be eligible for a second dose based on predefined criteria provided in a future amendment or separate study protocol.

Toxicities will be graded and documented according to the criteria described in the protocol.

All cumulative AEs occurring outside the DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the DSMB.

Dose-limiting Toxicities: Rationale and Criteria

The DLT definitions used in this study are informed by nonclinical studies of CTX310 and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas-9–based genome editing therapy. Adverse events that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

1. Any CTCAE grade ≥3 elevations in alanine aminotransferase (ALT) and aspartate aminotransferase (AST) that persist for >14 days and is assessed by the investigator as related to investigational product.
2. Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) that is assessed by the investigator as related to investigational product.
3. Any other CTCAE grade 3 laboratory abnormality that persists ≥7 days and is assessed by the investigator as related to investigational product.
4. Any other CTCAE grade 4 laboratory abnormality that is assessed by the investigator as related to investigational product.
5. Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product.

Study Eligibility

Inclusion Criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

Phase 1a and Phase 1b

1. Age of ≥18 and ≤75 years at the time of signing the informed consent.
2. Able to provide written informed consent.
3. Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>150 mg/dL [1.7 mmol/L]) and/or LDL-C (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for subjects with ASCVD) and/or non–HDL-C (>160 mg/dL [4.1 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L]) at screening, despite treatment. In Phase 1b cohort expansion part of the study, severe HTG is defined as TG levels of >500 mg/dL (5.65 mmol/L).
4. Subjects' lipid levels must be refractory to the maximal intensity or MTDs of standard of care lines of lipid-lowering therapies or combinations where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid or docosahexaenoic acid), bile acid sequestrants, and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9; alirocumab or evolocumab), for at least 12 weeks prior to screening.
5. Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation.
7. Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation except as required by the protocol.
8. Female subjects must be postmenopausal, defined as:
    - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
    - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male subjects must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in the protocol, from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.

Exclusion Criteria

To be eligible for entry into the study, the subject must not meet any of the exclusion criteria listed below:

Phase 1a and Phase 1b

1. Subjects with familial chylomicronemia syndrome (FCS) with <5% LPL activity, as documented in the medical history. If LPL activity testing is not documented, the subject with FCS will be excluded.
2. Evidence of liver disease, defined as:
   a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >2 × ULN, or
   b. Prothrombin time (international normalized ratio) >1.5 × ULN, or
   c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography.
3. Complete blood count: Neutrophils <1000 cells/μL (1.0×10^9/L); lymphocytes <500 cells/μL (0.5×10^9/L); hemoglobin <11 g/dL (110 g/L) for males, <10 g/dL (100 g/L) for females; or platelet count <100,000/μL (100×10^9/L).
4. Estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick or urine albumin to creatinine ratio of >300 mg/g.
6. Inadequate diabetes control, with glycosylated hemoglobin >9%.
7. History of alcohol or substance use disorder.
8. History of a significant coagulation disorder.
9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.1 mIU/L or >10 mIU/L).
10. Cardiac left ventricular ejection fraction <50% by echocardiogram.
11. Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm^2).
12. Peripheral pulse oximetry saturation of <90%.
13. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic of >90 mmHg, confirmed by a repeat measurement.
14. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
15. Acute coronary syndrome event within 24 weeks prior to Day 1.
16. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
17. Acute pancreatitis within 12 weeks prior to Day 1.
18. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).

19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for human immunodeficiency virus type 1 or type 2, hepatitis B virus (hepatitis B core antibody testing or hepatitis surface antigen or nucleic acid testing [NAT]), or hepatitis C virus (hepatitis C antibody testing or NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior malignancy within the past 5 years, or current malignancy (except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ and carcinoma in situ of the cervix or breast), or myeloproliferative disorder, or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, have an intact uterus and at least 1 ovary, and are less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
28. Administration of vaccines 30 days before CTX310 infusion.

For Phase 1b only

29. Treatment with evinacumab within 20 weeks prior to Day 1.

Statistical Methods

Sample Size

The study will initially enroll approximately 45 subjects to provide a preliminary evaluation of safety and efficacy of CTX310.

Analyses

The analysis sets are described in the statistical analyses section of the protocol.

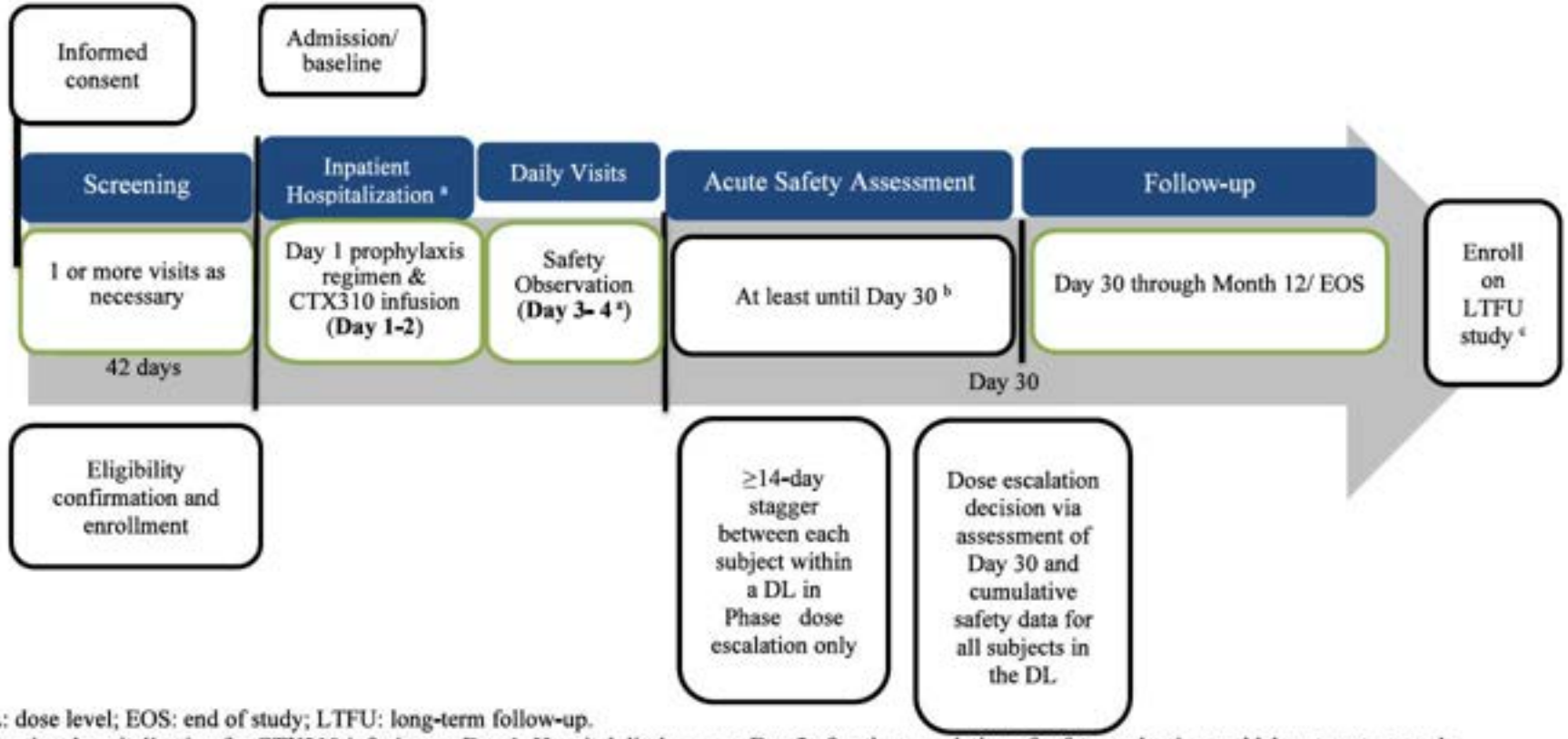
The safety and tolerability of CTX310 will be assessed in the safety analysis set using descriptive summaries. Summaries of AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each DL of CTX310 and overall. Summaries of AEs will focus on treatment-emergent adverse events (TEAEs). The incidence of TEAEs will be summarized by system organ class and preferred term, protocol-specified severity grade, and relation to CTX310. The incidence of DLTs, serious adverse events (SAEs), and AESIs will be also summarized. Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of clinically significant laboratory

abnormalities and other clinically significant safety measure abnormalities (e.g., ECG) will be summarized.

The preliminary efficacy of CTX310 will be assessed in the full analysis set using descriptive summaries. The percentage changes in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each CTX310 DL. Categorical summaries based on appropriate cutoff at selected time points, including 26 and 52 weeks after infusion, may be provided.

PK and PD data will be assessed descriptively and by exploratory modeling, as applicable.

1.2. Study Schema



DL: dose level; EOS: end of study; LTFU: long-term follow-up.

[a] Inpatient hospitalization for CTX310 infusion on Day 1. Hospital discharge on Day 2 after the completion of safety evaluation and laboratory tests and a minimum of 24 hours after the completion of the CTX310 infusion. Daily safety visits on Day 3 and 4. Inpatient hospitalization may be extended or following hospital discharge the subject may be readmitted or additional daily safety visits beyond Day 4 may be required at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

[b] For each DL during Phase 1a dose escalation, there will be a safety monitoring period of ≥14 days between the treatment of each subject and any subsequent subjects within the DL, or until the subject is clinically stable and all laboratory values (including liver function tests) have returned to <2 × baseline or to normal levels, whichever is later.

[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for roll over into a separate LTFU study for up to 15 years post-infusion

1.3. Schedule of Assessments

Table 1: Schedule of Assessments

		Treatment	Follow-up											
	Screening[1]	Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Eligibility and Other Assessments														
Informed consent	X													
Demographics, medical history	X													
History of pancreatitis events (for subjects with severe HTG)[4]	X													
Physical exam[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height, weight, BMI, waist to hip ratio	X													X
Vital signs[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver MRE or FibroScan[7]	X													
Liver MRI-PDFF or ultrasound[7]	X													X
Echocardiogram[8]	X[7]													
12-lead ECG[9]	X	X	X			X			X		X	X		X
Eligibility confirmation[10]	X													

Assessment	Screening[1]	Treatment	Follow-up											
		Inpatient Hospitalization[2]		Daily Safety Visits										
	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Treatment														
Pre-infusion prophylaxis regimen[11]	X	X												
CTX310 infusion[12]		X												
Safety Assessments														
Acute DLT assessment		X	X	X	X	X	X	X	X					
Adverse events	X													
Concomitant meds	X													
Laboratory Assessment (Local)[13]														
Serum chemistry	X	X	X	X	X	X	X	X	X		X	X	X	X
Urinalysis	X		X		X				X		X	X	X	X
Coagulation panel	X	X	X	X	X	X	X	X	X		X	X	X	X
Pregnancy test[14]	X													X
Hematology (CBC)	X	X	X	X	X	X	X		X			X		X
HbA1c	X											X	X	X
Thyroid function	X											X		X
eGFR (MDRD equation)	X								X			X		X
Viral serology	X													

Assessment	Screening[1]	Treatment		Follow-up										
		Inpatient Hospitalization[2]		Daily Safety Visits										
	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Cardiac biomarker test[13]		X												
Laboratory Assessments (Central)														
Genetic testing[15]	X													
Lipid panel[16]	X						X		X	X	X	X	X	X
Biomarkers (Plasma, Central)[17]														
ANGPTL3 levels[18]	X						X		X		X	X	X	X
PK studies[19]	X	X	X	X	X	X	X		X		X	X		X
Exploratory Biomarkers (Central)														
Immunogenicity[20]	X					X			X		X	X		X
Whole blood for storage[21]	X													
Plasma for storage[22]	X													
Serum[23]	X	X	X	X										

AESI: adverse event of special interest; ANGPTL3: angiopoietin-like 3; BMI: body mass index; Cas9: CRISPR-associated protein; CBC: complete blood count; CRISPR: clustered regularly interspaced short palindromic repeats; D or d: day; DLT: dose-limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; eLBW: estimated lean body weight; EOS: end of study; HbA1c: glycosylated hemoglobin; HTG: hypertriglyceridemia; LTFU: long-term follow-up; M: month; meds: medications; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI-PDFF: magnetic resonance imaging–protein density fat fraction; PK: pharmacokinetic; W: week.

[1] See Section 8.1.1.1 for detailed guidance. For the subject's convenience, if an assessment was performed before signing the ICF as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe. Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to

determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

[2] Subjects will be hospitalized on Day 1 for CTX310 infusion. Hospital discharge will occur a minimum of 24 hours post completion of CTX310 infusion and after Day 2 safety evaluations are complete and relevant laboratory tests have been reviewed. Subjects must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours completion of CTX310 infusion, or following hospital discharge, the subject may be readmitted or required to stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice. See Section 6.1.2 for discharge criteria and Section 8.1.1 for further description of study periods.

[3] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion (Section 8.1.1.5).

[4] Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment and during the screening period will be collected (Section 8.2.2).

[5] Complete physical exam required at screening, D1, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3).

[6] Vital signs: Blood pressure, heart rate, respiratory rate, oxygen saturation, temperature (Section 8.2.4). Subjects excluded from the study due to uncontrolled hypertension must have blood pressure measurements repeated at least 15 minutes later for confirmation. On Day 1 vital signs should be recorded at the following time points: prior to pre-infusion prophylaxis, prior to the infusion of CTX310, every 15 (± 5) minutes) during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital.

[7] Liver imaging: The same type of imaging should be used across all study visits. See Section 8.2.5.

[8] Transthoracic echocardiogram will be performed at screening. See Section 8.2.6 for details.

[9] Electrocardiogram: See Section 8.2.7. Day 1 ECG can be collected within 24 hours prior to Day 1 pre-infusion prophylaxis regimen.

[10] Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment. Also See Laboratory Assessments for Eligibility Confirmation (Section 8.2.8).

[11] Pre-infusion prophylaxis regimen: See Section 6.1.1.

[12] See Section 14.1 for eLBW calculation. See Section 6.1 for other details regarding CTX310 administration.

[13] See listings of laboratory assessments (Table 4 and Table 5) for details. Day 1 local laboratory assessments should be performed within 24 hours prior to the infusion of CTX310. Prior to the administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to monitor AESI (Section 9.3) and stopping rules (Section 10.2).

[14] At screening, all females must have a negative serum pregnancy test performed as per local standard. Serum or urine pregnancy test can be performed at EOS/M12 Visit. See Section 8.2.8, Table 4, and Section 8.2.9.

[15] The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.8 and Table 5 for details. Collection of a sample for genetic testing should not be repeated during rescreening, if applicable

[16] Lipid panel: See Section 8.2.8, Table 5 for listing of lipid panel components. Subjects on apheresis should have lipid levels sampled within 5 days prior to the procedure (pre-apheresis sample on the day of apheresis is also adequate). All lipid panels must be performed after a minimum 8 hour fast.

[17] Sponsor may request discontinuation of sample collections. Continue sample collection for all listed time points until instructed otherwise by the sponsor.

[18] Plasma samples will be obtained to assess ANGPTL3 levels (Section 8.4.2).

[19] PK testing: See Section 8.4.1: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (± 15 min) hours after completion of CTX310 infusion. On D2, D3, and D4 (24 [± 2], 48 [± 2], and 72 [± 2] hours post-infusion time points, respectively), a single sample will be collected. A single sample will also be collected for all other scheduled time points.

[20] Immunogenicity: See Section 8.3.

[21] Whole blood collection: Whole blood samples will be obtained at screening and stored (Section 8.4.3.1).

[22] Plasma for storage: See Section 8.4.3.2.

[23] Serum samples for exploratory biomarker assessments (e.g., cytokines): See Section 8.4.3.3: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (±15 min) hours after completion of CTX310 infusion. On D2 and D3 (24 [± 2] and 48 [± 2] hours post-infusion time points, respectively), a single sample will be collected.

2. INTRODUCTION

CTX310 is being developed by CRISPR Therapeutics AG (sponsor) for the treatment of subjects with refractory dyslipidemias with persistently elevated levels of TG and/or non– high-density lipoprotein cholesterol (HDL-C) and/or low-density lipoprotein cholesterol (LDL-C) and/or apolipoprotein B (ApoB). It is an in vivo gene editing therapy designed to target the gene for angiopoietin-like 3 (*ANGPTL3)*.

This first-in-human (FIH) study will evaluate the safety and tolerability of CTX310 in high-risk adult subjects with dyslipidemias refractory to available treatments.

2.1. Dyslipidemias

Dyslipidemias are among the most commonly detected and treated chronic conditions. They are characterized by abnormal levels of related lipoprotein species and abnormal serum levels of cholesterol, TG, or both. One of the most common clinical consequences of dyslipidemias is increased risk of atherosclerotic cardiovascular disease (ASCVD), which is associated with elevated levels of non–HDL-C (primarily LDL-C) and TG. With ASCVD remaining the leading cause of death and disability worldwide (Barquera et al., 2015), new treatment options to add to the current standard of care are clearly needed.

The management of dyslipidemias remains the cornerstone of cardiovascular disease (CVD) prevention. As reported by the American Heart Association (AHA) in 2021, 38% of adults (93.9 million) in the United States (US) had total cholesterol levels ≥200 mg/dL (5.2 mmol/L) from 2015 to 2018, with elevated levels of LDL-C (≥130 mg/dL (3.4 mmol/L)) reported in 29% of adults from 2013 to 2016 (Virani et al., 2021). Dyslipidemias involving elevated levels of LDL-C (hypercholesterolemia), triglyceride(s) (TG, hypertriglyceridemia [HTG]), or both also contribute to CVD and its associated risks, including type 2 diabetes, chronic kidney disease, and nonalcoholic fatty liver disease (Vijayaraghavan, 2010; Yang et al., 2021) . Additional clinical consequences associated with rare dyslipidemias such as severe elevations in TG include increased risk of pancreatitis.

The latest recommendations of Canadian, Australian, European, and American cardiological associations emphasize the role of increased levels of non–HDL-C and ApoB in evaluating the risk of CVD (Brett et al., 2021; Grundy et al., 2019; Mach et al., 2020; Pearson et al., 2021; Virani et al., 2021; Watts et al., 2021), rather than LDL-C and TG. Non–HDL cholesterol (i.e., total cholesterol – HDL-C) is the composite of LDL, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL), and lipoprotein(a) (Lp(a)). ApoB, the major structural protein in VLDL, IDL, LDL-C, and Lp(a), is a highly atherogenic apolipoprotein due to its arterial retention, resulting in plaque buildup in arterial walls over time (Sniderman et al., 2019). Although there is typically a good correlation between LDL-C and ApoB in calculating CVD risk, there is a discordance between the 2 parameters in approximately 20% of cases.

Therefore, non–HDL-C (indirectly) and ApoB (directly) provide a more accurate assessment of the total concentration of atherogenic particles, especially at higher TG concentrations, in non-fasting samples and in individuals with low LDL-C (Bergmann, 2010; Carr et al., 2019). The 2021 Canadian Cardiovascular Society (CCS) guidelines use either non–HDL-C or ApoB as the

preferred parameter for assessment of CVD risk. Achievement of treatment target values for ApoB and non–HDL-C have been modified from previous versions of CCS guidelines to accurately represent the same percentile equivalents as LDL-C for all recommended thresholds (Table 2) and inform the inclusion of non–HDL-C and ApoB in this study.

Table 2: ApoB and Non–HDL-C Threshold Selection

LDL-C		non–HDL-C		ApoB		
mmol/L	**mg/dL**	**mmol/L**	**mg/dL**	**mmol/L**	**mg/dL**	**g/L**
1.8	70	2.4	93	1.8	70	0.7
2.0	78	2.6	101	2.1	80	0.8
3.5	135	4.2	162	2.7	105	1.05
5	193	5.8	224	3.7	145	1.45

ApoB: apolipoprotein B; CCS: Canadian Cardiovascular Society; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.
Adapted from the 2021 CCS guidelines (Pearson et al., 2021).

Patients with dyslipidemias are typically treated with lipid-lowering therapies, which may include a statin, ezetimibe, lomitapide, icosapent ethyl, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (monoclonal antibodies and ribonucleic acid [RNA] inhibitor), and monoclonal antibody targeting ANGPTL3, where indicated and accessible. Despite all available treatments, only 45% of patients achieve target lipid levels suggested by AHA and American College of Cardiology (ACC) guidelines, especially patients who are at very high risk of cardiovascular events (Pearson et al., 2021; Rallidis et al., 2020), with approximately 50% of patients with ASCVD meeting the definition of high risk (An et al., 2020; Sajja et al., 2021).

Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes as described in Section 2.1.1 and Section 2.1.2, respectively.

2.1.1. Hypertriglyceridemia

Per the Endocrine Society Clinical Practice Guidelines and the National Cholesterol Education Program Adult Treatment Panel III, normal fasting TG levels can be defined as <150 mg/dL (1.7 mmol/L), borderline high as 150 to 199 mg/dL (1.7 to 2.3 mmol/L), high as 200 to 499 mg/dL (2.3 to 5.6 mmol/L), and very high or severe as >500 mg/dL (5.65 mmol/L).

Hypertriglyceridemia is separated into 2 populations: secondary causes related to disease, medications, or diet, and primary genetic syndromes or susceptibility (Rygiel, 2018). Elevated TG levels (>150 mg/dL [1.7 mmol/L]) are present in approximately 33% of adults in the US and are most commonly due to secondary causes rather than primary genetic syndromes (Oh and Trivette, 2020).

Acquired causes of HTG are most commonly due to medical conditions (e.g., diabetes mellitus, metabolic syndrome, central obesity, hypothyroidism, chronic kidney disease, autoimmune disorders), medications, and diet/lifestyle (e.g., alcohol use, physical activity (Rygiel, 2018).

Genetic causes of HTG may be characterized as multifactorial chylomicronemia syndrome (MCS) and familial chylomicronemia syndrome (FCS). MCS is a polygenic condition caused by heterozygous mutations in the lipoprotein lipase gene (*LPL*) or in the genes for apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*), lipase maturation factor 1 (*LMF-1*), or glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (*GP1HBP1*), or by multiple variants that are expressed in the presence of secondary factors (D'Erasmo et al., 2019). MCS is associated with a high CVD risk (Sarwar et al., 2007; Virani et al., 2021) and affects approximately 1 in 250 to 1 in 800 persons (Fan et al., 2020; Laufs et al., 2020; Paquette and Bernard, 2022). TG levels range from the upper level of normal to severe (>150 to 1999 mg/dL [1.7 to 22.6 mmol/L]).

FCS is a rare monogenic condition caused by the same homozygous or compound heterozygous mutations observed in MCS. Patients with FCS present at an earlier age, have severe levels of fasting TG and a higher incidence of acute pancreatitis. FCS is diagnosed based on fasting TG levels of ≥750 mg/dL (8.5 mmol/L) that do not respond to standard lipid-lowering therapy (Brahm and Hegele, 2015). As most FCS patients are either LPL-deficient or lack sufficient LPL activity, these patients will likely not benefit from *ANGPTL3*-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and, therefore, patients with FCS will be excluded from the study unless they have a documented medical history of ≥5% LPL activity.

The risk of acute pancreatitis is elevated with increasing levels of serum triglycerides. Severe hypertriglyceridemia ≥500 mg/dL is an established risk factor for acute pancreatitis (Gouni-Berthold et al., 2023). The risk of acute pancreatitis is approximately 5% for triglyceride levels >1000 mg/dL and 10% to 20% for levels >2000 mg/dL. Controlling triglyceride levels to <500 mg/dL can effectively prevent recurrences. Lipid-lowering therapies play a significant role in reducing the risk of recurrent acute pancreatitis in patients with elevated TG. Although TG levels close to normal may be preferable, levels <500 mg/dL represents a safe therapeutic target for prevention of recurrences(Scherer et al., 2014b).

The effect of LDL-lowering drugs such as statins, ezetimibe and PCSK9 inhibitors on TG levels in patients with MCS is usually modest (5% to 15% improvement). The main goal of treatment of MCS is to reduce TG concentration to <5.6 mmol/L (500 mg/dL) in order to prevent acute pancreatitis (Christian et al., 2012; Scherer et al., 2014b) followed by a secondary goal of reducing cardiovascular risk. The primary management of HTG consists of lifestyle modifications such as dietary fat restriction and reduced alcohol consumption, with the use of TG-lowering medications such as fibrates, omega-3 fatty acids, niacin, and icosapent ethyl, which can lower TG levels by 25% to 45% with limited impact on reductions in pancreatitis or CVD risk (Grundy et al., 2019). Intervention to prevent HTG in ASCVD is currently limited to icosapent ethyl, which was 25% effective at reducing relative risk for its primary study endpoint - a composite of cardiovascular death, nonfatal myocardial infarction, nonfatal stroke, coronary revascularization, and unstable angina (Bhatt et al., 2019; Virani et al., 2021).

Given the need for a significant improvement in TG-lowering therapies, based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of both LDL-C and TG.

2.1.2. Familial Hypercholesterolemia

Familial hypercholesterolemia is characterized by lifelong elevations in LDL-C and can be separated into 2 forms: homozygous familial hypercholesterolemia (HoFH) and heterozygous familial hypercholesterolemia (HeFH).

HoFH is a rare monogenic autosomal disorder with a prevalence of 1:300,000, and is characterized by significant elevations in LDL-C (Raal et al., 2020). Patients with HoFH may develop tendon xanthomas (lipid deposits) and can develop premature CVD, including heart attack and aortic valve disease, when they are teenagers or in their 20s. Without aggressive treatment, patients may die before age 30. The majority of HoFH (85% to 90%) is due to a biallelic deficiency or defect in the LDL receptor gene (*LDLR*). The remaining population have a defect in *ApoB* (loss-of-function [LOF] mutation) or *PCSK-9* (gain-of-function [GOF] or the rare autosomal recessive hypercholesterolemia (Sjouke et al., 2015)). LDL-C levels are elevated due to a failure to synthesize LDLR (receptor-negative), or defective binding or release at the lipoprotein receptor interface, resulting in the inability to clear LDL-C from circulation and giving rise to LDL-C levels of >400 mg/dL (10.3 mmol/L). Traditional lipid-lowering therapies, including high-dose statins, PCSK9 inhibitors, bile acid sequestrants, and ezetimibe, have little to modest activity in HoFH. Plasmapheresis or lipoprotein apheresis is used when available. The recent approval of an ANGPTL3-targeting antibody, evinacumab, provides a safe and effective option for patients with HoFH (Raal et al., 2020). When administered at 15 mg/kg on a monthly regimen, study subjects showed a 47% reduction in LDL-C compared to baseline, leaving significant room for improvement for LDL-lowering in this patient population. AHA guidelines recommend LDL-C levels <70 mg/dL (1.8 mmol/L) in patients at high risk for CVD. Compared with repeat dosing of a monoclonal antibody, CTX310 may offer a one-time treatment option for HoFH patients who require additional and significant LDL-C lowering.

HeFH is a common autosomal dominant disease, affecting approximately 1 in 250 people, with a significantly higher incidence in high-risk ASCVD (1:17) compared to the general population (Sturm et al., 2018). Similar to HoFH, the most common causes of HeFH are pathogenic variants of *LDLR*, which are responsible for 85% to 90% of genetically confirmed HeFH (Benn et al., 2016). Additional pathogenic variants of *ApoB* resulting in decreased binding of LDL to the LDLR, or GOF mutations in *PCSK9* result in increased destruction of LDLR and a >20-fold increase in ASCVD (Soutar and Naoumova, 2007). Although statins lower LDL-C by 18% to 55%, as many as 80% of statin-treated patients with established ASCVD fail to reach guideline-recommended target LDL-C levels (Marz et al., 2018). The addition of ezetimibe and PCSK9 inhibitors such as monoclonal antibodies and RNA inhibitors (inclisiran) to high-dose statin treatment lowers LDL-C by an additional ~15% to 50%, respectively (Grundy et al., 2019; Wright et al., 2021). Patients who are the least responsive to treatment are at highest risk of developing ASCVD. Evinacumab was evaluated in subjects with refractory hypercholesterolemia (LDL-C ≥100 mg/dL [2.6 mmol/L], or LDL-C ≥70 mg/dL [1.8 mmol/L] with ASCVD; (Rosenson et al., 2020). In this Phase 2 study, 70% to 80% of subjects had HeFH, approximately 60% were receiving statin therapy, 100% were receiving a PCSK9 inhibitor, and approximately 30% were receiving ezetimibe, reflecting a population of patients with refractory

hypercholesterolemia who may benefit from additional therapies designed to lower LDL-C. A reduction of LDL-C of up to 50% was achieved upon repeat dosing.

2.2. ANGPTL3

ANGPTL3 regulates plasma lipid levels through inhibition of LPL and endothelial lipase (EL). Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Pouwer et al., 2020). *ANGPTL3* LOF variants have been associated with decreased levels of TG, LDL-C, non-HDL-C, ApoB, and HDL-C, as well as a 41% lower risk of coronary artery disease (Dewey et al., 2017). Mechanistic studies indicate that ANGPTL3 inhibition leads to clearance of VLDL remnant particles, upstream of LDL formation, and that LDL-C lowering with ANGPTL3 inhibitors is independent of LDL receptor function (Adam et al., 2020; Reeskamp et al., 2021; Wu et al., 2020).

The LOF variants in *ANGPTL3* have been associated with decreased levels of both LDL-C and TG, and a 41% lower risk of coronary artery disease, with no pathologic manifestations despite the presence of low levels of HDL-C (Athyros et al., 2018; Calandra et al., 2017; Dewey et al., 2017; Stitziel et al., 2017; Tarugi et al., 2019).

Collectively, these data indicate that *ANGPTL3* is a valid therapeutic target to lower plasma LDL-C, non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who are at high risk of CVD. In vivo disruption of *ANGPTL3* in the liver using CTX310 may therefore provide clinical benefit in the study population with elevated lipid profiles selected for the FIH Phase 1 study.

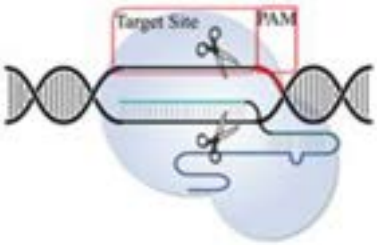
2.3. CRISPR Technology

CRISPR (clustered regularly interspaced short palindromic repeats) are found flanking foreign deoxyribonucleic acid (DNA) sequences in many bacteria and archaea. CRISPR are an important part of an adaptive bacterial defense system using RNA-guided DNA cleaving enzymes (Barrangou et al., 2007; Hale et al., 2009). The RNAs expressed from CRISPR sequences direct the sequence-specific binding of CRISPR-associated protein 9 (Cas9) nuclease. These key bacterial defense systems were adapted as a programmable RNA-directed CRISPR-Cas9 system for editing genomes (Jinek et al., 2012).

CRISPR-Cas9 systems can be directed by a complex of 2 distinct RNAs: crispr RNA (crRNA) and trans-activating crispr RNA (tracrRNA), or by a single-guide RNA (sgRNA) containing the crRNA and tracrRNA joined by a loop (Jinek et al., 2012). Delivery of Cas9 nuclease and sgRNA into a cell result in cleavage of the cell's genomic DNA at sequences specified by the sgRNA (Figure 1), leaving double-stranded breaks (DSBs). These DSBs are repaired by the cell's own DNA repair machinery. Nonhomologous end joining (NHEJ) is the predominant cellular repair pathway that is active during all phases of the cell cycle (Figure 2). However, NHEJ is an imprecise mechanism for DNA repair and often results in insertions or deletions (indels) at the cut site that can lead to gene disruption and potential LOF. The NHEJ repair pathway can be co-opted to insert DNA sequences at targeted Cas9-sgRNA cut sites in nondividing cells, a process referred to as homology-independent insertion (Figure 2). Homology

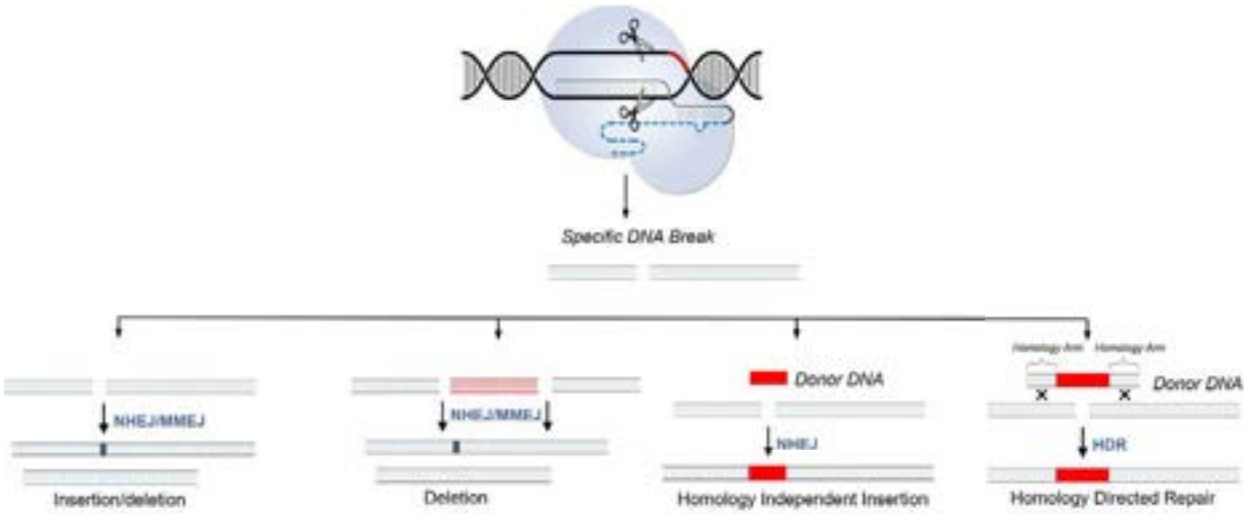
directed repair (HDR) is the second most common repair mechanism for DSBs but, unlike NHEJ, is only active during late S and G2 phases of the cell cycle. HDR relies on the presence of a homologous repair template (Figure 2) and, as a result, DNA is often repaired faithfully with no indel formation. In cycling cells, HDR in the presence of genomic sequences containing homology arms can be used to correct mutations or introduce novel sequences at specific cut sites.

Figure 1: Schematic of the CRISPR-Cas9 Complex



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; crRNA: crispr RNA; PAM: protospacer adjacent motif; RNA: ribonucleic acid; sgRNA: single-guide RNA; tracrRNA: trans-activating crispr RNA.
CRISPR-Cas9 complex containing a sgRNA wherein the crRNA and tracrRNA are joined by a linker loop.

Figure 2: CRISPR-Cas9–mediated Genome Editing Strategies



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; DNA: deoxyribonucleic acid; MMEJ: microhomology-mediated end joining; NHEJ: nonhomologous end joining.

Target sites for CRISPR-Cas9 systems are distributed throughout the genome. A requirement is that the target sequence, homologous to the 5' end of the sgRNA and typically 20 nucleotides long, is followed by a protospacer adjacent motif (PAM) sequence (Horvath et al., 2008; Mojica et al., 2009; Shah et al., 2013). For *Streptococcus pyogenes* CRISPR-associated protein 9 (SpCas9), the PAM is any nucleotide followed by a pair of guanines (denoted as NGG).

The Cas9 nuclease searches for the PAM site and adjacent sequence matching the sgRNA 5' end before cleaving the DNA. Target site specificity results from a required combination of the site matching the sgRNA adjacent to a PAM site: the Cas9 nuclease does not bind to sequences without being complexed to a matching sgRNA, and the Cas9 nuclease and sgRNA will not bind or cut unless the target sequence is adjacent to the PAM.

CTX310 utilizes CRISPR-Cas9 to selectively cut exon 1 of *ANGPTL3*, which is specifically expressed in the liver. The resulting indels via NHEJ-mediated repair (active in nondividing hepatocytes) lead to small frameshift mutations and a premature stop codon, resulting in protein knockdown. The consequence of the gene editing is the reduction of ANGPTL3 protein secreted into circulation.

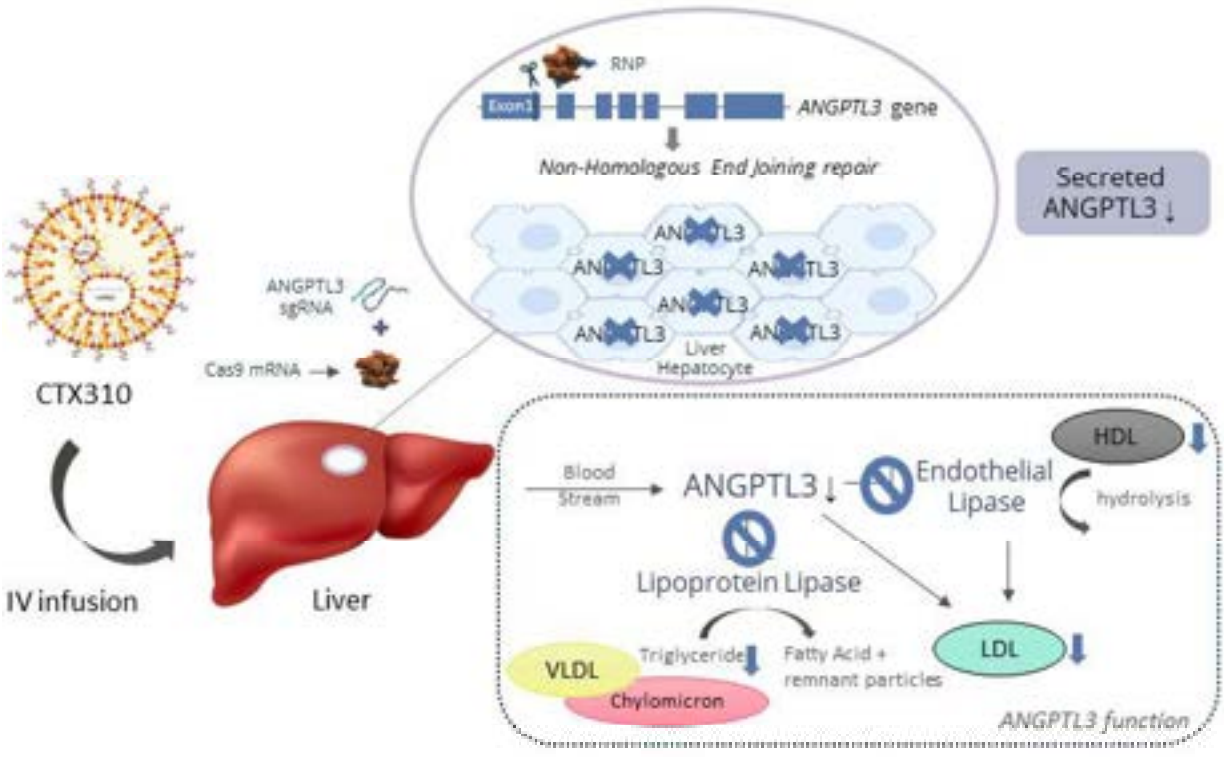
2.4. CTX310

CTX310 is a lipid nanoparticle (LNP) formulation of CRISPR-Cas9 components for in vivo gene editing of the target gene *ANGPTL3*.

The CTX310 drug product (DP) is a sterile formulation that consists of 2 drug substances (DSs): messenger ribonucleic acid (mRNA) encoding SpCas9, and [redacted] sgRNA targeting the gene of interest with a defined mass ratio encapsulated in an LNP, both of which will be individually manufactured prior to coformulation into DP. The DSs and DP will be manufactured and stored according to Good Manufacturing Practice. The first DS is a capped and polyadenylated SpCas9 mRNA containing N1-methylpseudouridine. The second DS, sgRNA, is a 100 nucleotide–long single-stranded oligonucleotide. The DP is an LNP encapsulating the 2 DSs and is composed of 4 lipid components: a cationic lipid, a polyethylene glycol lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, and cholesterol. The DP LNPs have an average size of ~60 nm and range in size from approximately 50 to 100 nm.

The mechanism of action for CTX310 is the disruption and reduction of the ANGPTL3 biological pathway (Figure 3). Nonclinical data supporting the clinical use of CTX310 are summarized in the Investigator Brochure.

Figure 3: CTX310 Mechanism of Action



ANGPTL3: angiopoietin-like 3; Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; EL: endothelial lipase; HDL: high-density lipoprotein; IV: intravenous; LDL: low-density lipoprotein; LNP: lipid nanoparticle; LPL: lipoprotein lipase; mRNA: messenger ribonucleic acid; NHEJ: nonhomologous end joining; RNA: ribonucleic acid; RNP: ribonucleoprotein; sgRNA: single-guide RNA; SpCas9: *Streptococcus pyogenes* CRISPR-associated protein 9; TG: triglyceride(s); VLDL: very low-density lipoprotein.

CTX310 is delivered via IV infusion. Similar to other LNPs, CTX310 is expected to be taken up by liver hepatocytes. After uptake, CTX310 escapes from endosomes and releases encapsulated SpCas9 mRNA and sgRNA into cytosol. SpCas9 mRNA is translated to SpCas9 protein, which then forms an RNP complex with sgRNA; this RNP complex shuttles into the nucleus and binds the target sequence. The RNP complex cuts at exon 1 of the *ANGPTL3* locus and introduces frameshift mutations after NHEJ repair. This leads to the knockdown of ANGPTL3 protein expression and reduced secretion from hepatocytes into circulation. The dotted box portrays the role of ANGPTL3 in lipoprotein metabolism. ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; EL hydrolyzes phospholipids, mainly on HDL particles. ANGPTL3 also affects LDL levels through LDL receptor-dependent and -independent (via EL) pathways. Knockdown of ANGPTL3 de-represses the activity of these lipases and leads to reduction of TG and LDL levels.

2.5. Study Rationale

A one-time in vivo disruption of *ANGPTL3* in the liver using CTX310 may provide clinical benefit in patients with dyslipidemia with elevated levels of TG and/or non–HDL-C (including LDL-C) and/or ApoB that are refractory to current treatments, and where compliance to adherence with lifelong medications and optimal lifestyle continue to remain a challenge.

2.5.1. Rationale for Targeting *ANGPTL3*

The sponsor is developing a one-time gene editing therapy for patients with dyslipidemias who have responded inadequately to maximum tolerated doses (MTDs) and adequate duration of currently available treatments and have not achieved the target lipid levels recommended by current guidelines (i.e., who are refractory). The gene editing therapy utilizes CRISPR-Cas9 to specifically target and disrupt *ANGPTL3*, which encodes a regulator of lipoprotein metabolism expressed in the liver (Conklin et al., 1999) and has emerged as a therapeutic target for patients with mixed dyslipidemias. As described in Section 2.2, ANGPTL3 has been shown to inhibit activity of LPL, the main enzyme involved in hydrolysis of TG-rich lipoproteins, and EL, which hydrolyzes HDL phospholipids, and therefore increases TG and other lipids (Kersten, 2021; Shimamura et al., 2007; Shimizugawa et al., 2002). Decreased ANGPTL3 levels have been shown to exhibit higher LPL activity and thus reduced levels of TG (Christopoulou et al., 2019). *ANGPTL3* inhibition can also lead to efficient clearance of VLDL remnant particles via activation of EL in an LDLR-independent mechanism, leading to reduction in LDL-C, non-HDL-C, and ApoB levels (Adam et al., 2020; Rosenson et al., 2020).

Large-scale genetic studies in humans show LOF variants of *ANGPTL3* have low levels of TG and LDL-C and decreased risk of ASCVD (Dewey et al., 2017; Helgadottir et al., 2016) despite low levels of HDL-C (Minicocci et al., 2012; Musunuru et al., 2010; Stitziel et al., 2017). In addition, clinical studies targeting *ANGPTL3* by lowering or inactivating through antisense oligonucleotide or monoclonal antibody treatments have demonstrated efficacy in subjects with various forms of dyslipidemia to markedly reduce plasma LDL-C and TG levels (Graham et al., 2017; Raal et al., 2020; Rosenson et al., 2020; Watts et al., 2019). *ANGPTL3* inhibition also substantially lowers ApoB levels. Mendelian randomization analyses have shown this inhibition has been shown to proportionally decrease risk of CVD (Ference et al., 2019). Together, these studies indicate that *ANGPTL3* is a valid therapeutic target to lower plasma non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who remain at high risk of CVD.

2.5.2. Rationale for the Study Population

Based on the nonclinical understanding of and emerging clinical data from ANGPTL3-directed therapies, and a high unmet need in a treatment-refractory population at high risk for cardiovascular events, this Phase 1 study will include subjects with or without ASCVD who have monogenic or polygenic refractory dyslipidemias, including MCS, HoFH, HeFH, and other HTG/hypercholesterolemia syndromes of undetermined etiologies (Section 2.1).

Based on data from non– Good Laboratory Practice (GLP) and GLP toxicology studies in non-human primate (NHP) models in which non-adverse, dose-dependent transient elevations in liver function tests (LFTs) were observed, eligibility criteria for the clinical study will exclude

subjects with underlying liver fibrosis or past infections, alcohol abuse, compromised liver function, or co-morbidities that may compromise liver function. As the liver is the main target organ for CTX310, subjects with coagulation disorders or low platelet counts are also excluded. Subjects who do not meet adequate organ function criteria (e.g., for renal, lung, and cardiac function) are excluded from participating in the Phase 1 study.

As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema based on estimated lean body weight (eLBW), which takes into account gender and height rather than total body weight alone, was selected to reduce the risk of overdosing subjects with higher body mass index (BMI).

Due to the unknown risk of on-target editing in tissues other than the liver, including reproductive organs, women of childbearing potential will be excluded from this study, and male subjects enrolled in the study must agree to use highly effective method(s) of contraception from study consent through 12 months after CTX310 infusion, until additional nonclinical data are available to reassess the eligibility criteria (see Section 4).

The study considers the balance of risk and potential benefit for subjects in this FIH study of CTX310.

2.5.3. Rationale for Lipid Cutoff Values for Inclusion

Per AHA and CCS guidelines (Grundy et al., 2019; Pearson et al., 2021), subjects at high risk of CVD (i.e., LDL-C ≥100 mg/dL (2.6 mmol/L) for subjects without ASCVD or ≥70 mg/dL (1.8 mmol/L) for subjects with established ASCVD) are recommended for this study.

Similarly, subjects with non–HDL-C levels of ≥160 mg/dL (4.1 mmol/L) or ApoB levels of ≥100 mg/dL (2.6 mmol/L), identified as at high risk of CVD per CCS guidelines (Pearson et al., 2021), are also recommended for this study.

Triglyceride levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al., 2020; Watts et al., 2021) and are recommended for inclusion in the Phase 1a part of the study. Severe HTG, defined as TG levels of >500 mg/dL (5.65 mmol/L), is a risk factor for ASCVD and is recommended for inclusion in Phase 1b (Gouni-Berthold et al., 2023; Gurevitz et al., 2024). Analyses of the characteristics and prevalence of chronic conditions by TG levels have shown that these conditions and multiorgan disease are common at higher TG level, with a substantially increased risk of pancreatitis associated with this condition (Gouni-Berthold et al., 2023; Gurevitz et al., 2024; Scherer et al., 2014b).

2.5.4. Nonclinical Data with CTX310

In NHP studies, a single dose of CTX310 resulted in significant and sustained reductions in TG levels in a dose-dependent manner, and in a mouse *LDLR* knockout model, a mouse surrogate of CTX310 resulted in significant lowering of LDL-C. Together, these nonclinical data, summarized in the Investigator's Brochure, support the use of CTX310 as a one-time treatment to lower atherogenic lipids.

2.5.5. Clinical Data with CTX310

Preliminary data from DL1 (0.1 mg/kg eLBW) and DL2 (0.3 mg/kg eLBW) indicate a favorable safety profile. CTX310 was well-tolerated with no serious adverse events (SAEs) or serious adverse reactions (SARs), and no treatment-emergent Grade 3 or higher adverse event (AE). No clinically relevant abnormal coagulation findings, or dose dependent liver enzyme elevations higher than grade 1 were observed (data on file). All treatment-emergent adverse events (TEAEs) were mild to moderate in severity. The benefit-risk profile for CTX310 remains acceptable for continued clinical development.

3. STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives	Primary Endpoints
To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of DLTs and frequency of AEs
Secondary Objectives	**Secondary Endpoints**
To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non-HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
To further characterize the safety of CTX310	• Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline
Exploratory Objectives	**Exploratory Endpoints**
To identify changes associated with CTX310 that may indicate or predict clinical response, immunogenicity, safety, or PD activity	• Percentage change in FFA levels over time compared to baseline. • Change in fatty liver disease. • Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required). • For Phase 1b only Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG

ADA: anti-drug antibody; AE: adverse event; AESI: adverse event of special interest; [redacted] ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; DLT: dose-limiting toxicity; FFA: free fatty acid; HDL-C: high-density lipoprotein cholesterol; HTG: hypertriglyceridemia; GLDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; PD: pharmacodynamic(s); PK: pharmacokinetics; TEAE: treatment-emergent adverse event; TG: triglyceride(s).

4. SUBJECT ELIGIBILITY

4.1. Inclusion Criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

Phase 1a and Phase 1b

1. Age of ≥18 and ≤75 years at the time of signing informed consent.
2. Able to provide written informed consent.
3. Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>150 mg/dL [1.7 mmol/L]) and/or LDL-C (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for subjects with ASCVD) and/or non–HDL-C (>160 mg/dL [4.1 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L]) at screening, despite treatment. In Phase 1b cohort expansion part of the study, severe HTG is defined as TG levels of >500 mg/dL (5.65 mmol/L).
4. Subject lipid levels must be refractory to the maximal intensity or MTDs of standard of care lines of lipid-lowering therapies or combinations where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least 12 weeks prior to screening.
5. Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation.
7. Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change in frequency during the study participation except as required by the protocol.
8. Female subjects must be postmenopausal, defined as:
   - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
   - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male subjects must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in Section 14.2 , from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.

4.2. Exclusion Criteria

To be eligible for entry into the study, the subject must not meet any of the exclusion criteria listed below:

Phase 1a and Phase 1b

1. Subjects with FCS with <5% LPL activity, as documented in the medical history. If LPL activity testing is not documented, the subject with FCS will be excluded.
2. Evidence of liver disease, defined as:
   a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >2 × ULN, or
   b. Prothrombin time (international normalized ratio [INR]) >1.5 × ULN, or
   c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography (MRE).
3. Complete blood count: Neutrophils <1000 cells/μL (1.0×10^9/L); lymphocytes <500 cells/μL (0.5×10^9/L); hemoglobin <11 g/dL (110 g/L) for males, <10 g/dL (100 g/L) for females; or platelet count <100,000/μL (100×10^9/L).
4. Estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick or urine albumin to creatinine ratio of >300 mg/g.
6. Inadequate diabetes control, with glycosylated hemoglobin >9%.
7. History of alcohol or substance use disorder.
8. History of a significant coagulation disorder.
9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.1 mIU/L or >10 mIU/L).
10. Cardiac left ventricular ejection fraction (LVEF) <50% by echocardiogram.
11. Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm^2).
12. Peripheral pulse oximetry saturation of <90%.
13. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic >90 mmHg confirmed by a repeat measurement.
14. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
15. Acute coronary syndrome event within 24 weeks prior to Day 1.
16. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
17. Acute pancreatitis within 12 weeks prior to Day 1.

18. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for human immunodeficiency virus (HIV) type 1 or type 2, hepatitis B virus (hepatitis B core antibody or hepatitis B surface antigen or NAT), or hepatitis C virus (hepatitis C antibody testing or NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior malignancy within the past 5 years, or current malignancy (except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ and carcinoma in situ of the cervix or breast), or myeloproliferative disorder, or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, have an intact uterus and at least 1 ovary, and are less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
28. Administration of vaccines 30 days before CTX310 infusion.

For Phase 1b only

29. Treatment with evinacumab within 20 weeks prior to Day 1.

5. STUDY DESIGN

5.1. Investigational Plan

This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll approximately 45 subjects 18 to 75 years (inclusive) of age who have dyslipidemias with persistently high levels of TG and/or non–HDL-C (including LDL-C) and/or ApoB above the thresholds recommended by ACC and AHA guidelines despite MTDs of available lipid-lowering treatments, diet, and lifestyle modifications (i.e., refractory to indicated and available treatments; see Inclusion Criteria in Section 4.1).

This Phase 1 study will include subjects with monogenic or polygenic refractory dyslipidemias, with or without ASCVD, that encompass HTG and/or hypercholesterolemia syndromes, as described in Section 2.5.2. The study is divided into 2 parts (Figure 4): Phase 1a dose escalation followed by Phase 1b disease-specific cohort expansion. Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D) (Section 5.4.2). The FD will be a dose associated with optimal biological efficacy, as determined by the intended decrease in ANGPTL3 and key lipid levels with minimum toxicity. Phase1b will confirm the RP2D.

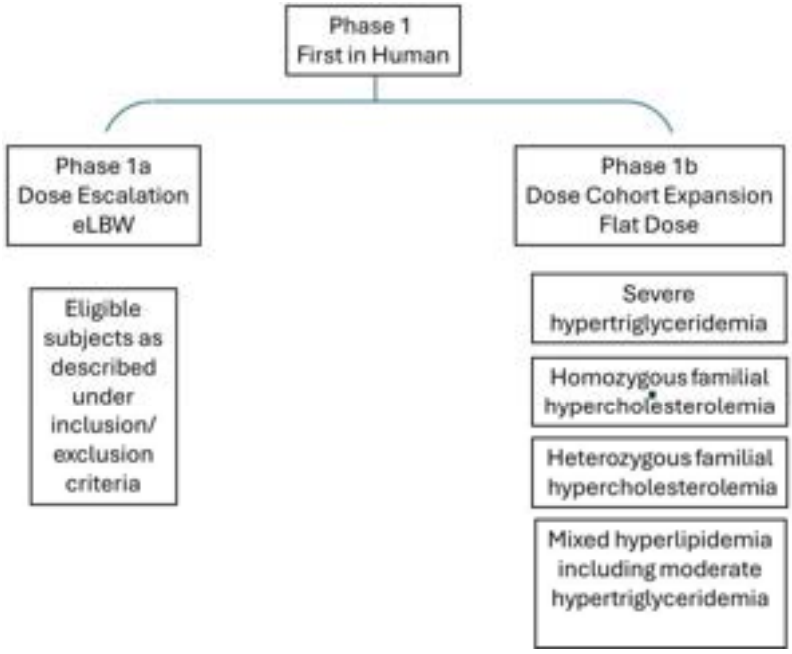
The Phase 1a dose escalation part of this study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:

- Multifactorial chylomicronemia syndrome (MCS).
- Homozygous familial hypercholesterolemia (HoFH).
- Heterozygous familial hypercholesterolemia (HeFH)
- Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).
- Other HTG/hypercholesterolemia syndromes of undetermined etiologies.

The Phase 1b disease-specific cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD that encompass HTG and/or familial hypercholesterolemia syndromes in the following disease-specific cohorts:

- Severe HTG (>500 mg/dL triglycerides)
- HoFH
- HeFH
- Mixed hyperlipidemias (including moderate HTG)

Figure 4: Study Design



In the Phase 1a, the majority of subjects enrolled are expected to have HeFH or be of polygenic background due to the high prevalence of HeFH and polygenic hypercholesterolemia and HTG (Goldberg and Chait, 2020; McGowan et al., 2019; Sturm et al., 2018). Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing CVD risk.

Subjects will be asked to continue to take their baseline lipid-lowering medications in the same doses throughout the study; adequate washout periods between the medications and CTX310 infusion have been included, when relevant.

Three to 6 subjects will be enrolled in each of the dose levels (DLs): 0.1, 0.3, 0.6, 0.8, 1.0 and 1.2 mg/kg eLBW of total RNA in the LNP formulation. Within each DL, a minimum 14-day stagger between dosing of CTX310 to each subject is required. Dose escalation will follow the criteria described in Section 5.4.

Following completion of Phase 1a eLBW-based dose escalation, the SRC will review the totality of safety and clinical activity data to delineate an FD for Phase 1b or anticipated RP2D. The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.

The FD will be applied to the Phase1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase1a. Phase 1b will confirm the RP2D. An additional FD may be evaluated in each expansion cohort if the safety or activity

profile of the first FD is inadequate and will not exceed the equivalent highest dose evaluated during Phase 1a.

Each subject will receive a single intravenous (IV) dose of CTX310 on Day 1 and will be hospitalized for a minimum of 24 hours after completion of CTX310 infusion (or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment). Following hospital discharge, subjects may be readmitted, if needed, for safety assessment. All subjects will be closely monitored post-infusion for AEs defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All subjects will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Subjects will be required to stay within 1 hour of the infusion site to enable daily (and as needed) clinic visits for safety assessment until completion of Day 4 visit (or longer at the discretion of the investigator for safety assessment).

After CTX310 infusion, subjects will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on ANGPTL3 levels and lipid profile. All subjects who receive CTX310, including those who discontinue early and those who complete the study, will be asked to consent to roll over into a separate long-term follow-up (LTFU) study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events.

At each Phase 1a dose escalation DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next DL and to Phase 1b disease-specific cohort expansion. Based on analysis of the totality of clinical data and the endorsement of the SRC, the sponsor will declare the RP2D.

5.2. Number of Study Subjects

Approximately 69 eligible subjects will be enrolled in the study.

Phase 1a Dose Escalation: Approximately 21 subjects.

Phase 1b Cohort Expansion: Approximately 48 subjects (up to 12 subjects per disease-specific cohort).

5.3. Study Duration

As illustrated in the study schema (Section 1.2), each subject will undergo the following stages:

1. Screening: Up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All subjects will be monitored for safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: All subjects will be asked to roll over to a separate LTFU study (Section 8.1.1.5).

5.4. Dose Escalation and Disease-specific Cohort Expansion

5.4.1. Dose Escalation Methodology

The doses in Table 3 are proposed for evaluation in the 4 planned dose escalation (Phase 1a) levels in the study, with a minimum of 3 and a maximum of 6 evaluable subjects per DL.

The dose rationale based on the nonclinical studies in NHPs is described in Section 5.5.

Table 3: Dose Escalation of CTX310

Dose Level*	Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6
4	0.8[2]
5	1.0[3]
6	1.2[4]

DL: Dose Level; eLBW: estimated lean body weight; RNA: ribonucleic acid; SRC: Safety Review Committee.
* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose.
[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW.
[2] Following review of clinical data by the sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.
[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.
[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 subjects will be treated at each DL depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in LFTs after dosing with CTX310 resolved within 14 days, the dosing between each subject within a DL will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs) have returned to <2 × baseline or to normal levels, whichever is later. If the safety evaluation of a subject is acceptable, the next subject in the DL may be dosed.

Dose escalation may proceed when all subjects in the preceding DL have completed dosing, the last subject has completed ≥30-day safety evaluation, and the cumulative safety data of all treated subjects at that DL demonstrates an acceptable safety profile, as determined by the SRC.

The SRC will review all available cumulative safety and clinical activity data when the DLT observation period ends for the last subject enrolled in each DL and will be responsible for making dose escalation decisions.

Throughout dose escalation, for cases in which a dose had been cleared in a DL and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to

enroll an additional number of subjects for a total of up to 6 at the current DL to gather additional safety data.

The sponsor, in conjunction with the investigators and the SRC, will decide whether to classify an event occurring within the first 30 days following infusion as a DLT.

Dose escalation will be performed according to the following rules:

- If 0 of 3 subjects experience a DLT, escalate to the next DL.
- If 1 of 3 subjects experiences a DLT, expand the current DL to 6 subjects.
  - If 1 of 6 subjects experiences a DLT, escalate to the next DL.
  - If ≥2 of 6 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- If ≥2 of 3 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 or any optional de-escalation DL as specified in Table 3, de-escalate to previous DL or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- No dose escalation is planned beyond the highest DL listed for the study (Table 3). Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones in Table 3, may be added during the course of dose escalation to support the identification of a safe and effective dose.

Toxicities will be graded and documented per criteria described in Section 9.

All cumulative AEs occurring outside the acute DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the independent DSMB.

5.4.2. Optimal Biologic Dose Definition

The OBD is the lowest dose associated with biological efficacy, as determined by intended decrease in ANGPTL3 levels, with minimum toxicity.

5.4.3. Dose-limiting Toxicity Rationale and Definitions During Phase 1a and Phase 1b

The DLT definitions used in this study are informed by nonclinical studies of CTX310, and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021). AEs that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

1. Any CTCAE grade ≥3 elevations in ALT and AST that persists for ≥14 days and is assessed by the investigator as related to investigational product.
2. Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (INR) that is assessed by the investigator as related to investigational product.

3. Any other CTCAE grade 3 laboratory abnormality that persists ≥7 days and is assessed by the investigator as related to investigational product.
4. Any other CTCAE grade 4 laboratory abnormality that is assessed by the investigator as related to investigational product.
5. Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product.

Subjects must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable subject (i.e., a subject that has been administered CTX310 and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable subjects are required per DL.

An adequate interval will be applied between current lipid-lowering treatments a subject is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities (Section 6.5.1).

Note: Any subject who experiences a DLT will be considered evaluable. Data for all subjects who receive CTX310 will be part of the safety analysis set.

5.4.4. Disease-specific Cohort Expansion

Following completion of eLBW-based dose escalation (Table 3), analysis of PK/PD and safety data, and approval by the SRC, a FD of CTX310 may be evaluated in a Phase 1b cohort expansion in 4 disease-specific cohorts.

The SRC will endorse the RP2D based on the review of the totality of data including clinical activity and safety of the FD for disease-specific groups. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future protocol amendment or separate study protocol.

5.5. CTX310 Dose Rationale

5.5.1. Dose Escalation in Phase 1a

The clinical study with CTX310 will utilize the same LNP formulation used in the CTX310 nonclinical NHP studies, allowing direct correlations between NHP dose and the expected safety and efficacy in human subjects. As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema is based on eLBW, which takes into account sex and height rather than total body weight alone, and, therefore, was selected to reduce the risk of overdosing obese and overweight subjects.

The FIH starting dose of CTX310 is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the NHP GLP safety and toxicology study (see Section 2.5.4 and refer to the CTX310 Investigator's Brochure). The initial starting dose of 0.1 mg/kg of CTX310 refers to the total RNA dose that is based on an anticipated NOAEL of 1 mg/kg. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg. The emerging clinical

data on systemically infused LNP-associated therapeutics demonstrate a relatively safe profile (Adams et al., 2018; Coelho et al., 2013; Gillmore et al., 2021). A 3-fold safety factor was initially proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies (Barros and Gollob, 2012).

Clinical exposure data available to date indicate that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Based on available clinical CTX310 PK from dose levels 1 through 3 (n=9) (data on file), the mean dose normalized $AUC_{0\text{-}Tlast}$ of [redacted] and [redacted] was 3,803,333 ± 1,933,384 and 825,000 ± 241,886 (hr*ng/mL)/(mg/kg), respectively. This exposure is approximately the same as the mean dose normalized $AUC_{0\text{-}Tlast}$ in the pivotal NHP GLP Toxicity study ([redacted]: 3,158,889 ± 990,153; [redacted]: 934,000 ± 30,199 (hr*ng/mL)/(mg/kg); Study 3359-006). This exposure data indicates that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Thus, scaling between NHPs and humans may support a human equivalent NOAEL of 1.0 mg/kg and toleration of 2.0 mg/kg. With gradual increments in DLs, dose escalation is expected to proceed from 0.1 to 1.2 mg/kg. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data.

5.5.2. Disease-specific Cohort Expansion with Flat Dose in Phase 1b

Due to the primary distribution of the LNP-mediated CTX310 delivery to the liver, evaluation of safety and clinical activity of FD of CTX310 is considered appropriate and consistent with other liver-targeted genetic medicines that are administered at a FD (Bakris et al., 2024; Coelho et al., 2023; Desai et al., 2023; Young et al., 2023); (Fontana et al., 2024; NCT06128629; Ray et al., 2020; Srivastava et al., 2023). Earlier siRNAs (givosiran, lumasiran) initially relied on weight-based dosing (Balwani et al., 2020; Garrelfs et al., 2021) with little difference in efficacy noted regardless of weight. Similarly, in vivo gene editors that target hepatocytes using LNP vehicles likewise began development with weight-based dosing (Gillmore et al., 2021), but transitioned to flat-dosing in later phases (NCT06128629) and with newer targets (Longhurst et al., 2024). A similar strategy is planned for Phase 1b, where PK, safety (LFTs) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and LDL) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts.

6. STUDY TREATMENT

6.1. Administration of CTX310

Subjects will receive a single IV infusion on Day 1, administered under medical supervision during inpatient hospitalization. The date and time of the dose administered will be recorded in the source documents and in the electronic case report form (eCRF).

Infusion of the study drug will be slowed or stopped in the event of an infusion-related reaction (IRR; Section 7.1.1).

Prior to the administration of CTX310 on Day 1, investigator should confirm the ability of the subject to receive the infusion (see Table 1) by ensuring:

- No significant change in clinical status since screening.
- No new, clinically significant findings seen on physical exam, vital signs or ECG.
- No AST, ALT, total bilirubin >2 × ULN and PT (INR) >1.5 × ULN.

Prior to the administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor (see Table 1).

If the infusion is delayed for more than 30 days, the subject will be replaced if deemed necessary for dose escalation decisions.

6.1.1. Pre-infusion Prophylaxis

On Day -1, i.e., the evening prior to CTX310 administration on Day 1, an infusion prophylaxis regimen will be administered to subjects as follows:

- Oral steroid (e.g., dexamethasone approximately 10 mg or equivalent)

On Day 1, within 1 to 2 hours prior to the administration of study drug, an infusion prophylaxis regimen will be administered to subjects as follows:

- IV steroid (e.g., dexamethasone 10 mg or equivalent);
- IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent) or oral H1 blocker (e.g., cetirizine 10 mg or equivalent); and
- IV or oral H2 blocker (e.g., famotidine 20 mg or equivalent).
- Optional oral acetaminophen/paracetamol (500 mg to 1000 mg)

6.1.2. CTX310 Post-infusion Monitoring

Following completion of study drug administration, subjects will be observed as inpatients for minimum of 24 hours, or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment. Subjects must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours post completion of CTX310 infusion, or the subject may be readmitted or required to

stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

Safety and clinical laboratory evaluations, and collection of blood and urine samples will be performed per the schedule of assessment (Table 1). Repeat laboratory evaluations may be required for assessment of AESI/ SAE or to meet study stopping criteria (see Section 9.3 and Section 10.1). All AEs and concomitant medications will be recorded.

Inpatient hospitalization for observation may be extended at the discretion of the investigator to follow and manage AEs as needed.

Subjects will be discharged from the study site when they meet the following criteria:

1. Are clinically stable as per the investigator's judgement.
2. Day 2 safety assessments have been performed and relevant laboratory results have been reviewed.
3. The frequency of monitoring of laboratory AEs can be handled in the outpatient setting (schedule of assessments Table 1 and Section 7.1.2).
4. Subjects are aware of contact information in case of an emergency and agree to remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit.

6.2. Investigational Product Preparation, Handling, Storage, and Accountability

CTX310 DP will be provided as a frozen liquid formulation consisting of 300 mM sucrose in phosphate buffered saline at a target concentration of 2.0 (± 0.4) mg/mL total RNA. CTX310 must be stored frozen at ≤-60°C in a glass vial until time of use. The DP will be stored onsite, thawed, and formulated immediately prior to administration. Refer to the Pharmacy Manual for detailed instructions on preparation, storage, handling, and administration of CTX310.

6.2.1. Investigational Product Accountability

The investigator and sponsor are responsible for accountability and traceability of CTX310 clinical supply.

The investigator will ensure that CTX310 is used in accordance with this protocol and the Pharmacy Manual. Detailed accountability records indicating CTX310 inventory at each clinical site, use by each subject, and disposal will be maintained by the clinical sites. To maintain compliance, the sponsor or its designee will review CTX310 clinical supply accountability records at the clinical sites on an ongoing basis during monitoring visits.

Instructions for destruction of all excess and expired material containing CTX310 will be provided directly by the sponsor. Destruction will be adequately documented and reviewed regularly by the sponsor or its designee and the investigator.

6.3. Comparator Product

This is a single-arm study with no comparator.

6.4. Measures to Minimize Bias: Randomization and Blinding

This is a single-arm, open-label study. Masking is not applicable. Randomization is not used in this study.

6.5. Prior and Concomitant Medications

All medications taken within 30 days before the signing of the informed consent form (ICF) will be recorded. All concurrent therapies, including prescription and nonprescription medications, must be recorded from the date of signed informed consent through 12 months after CTX310 infusion.

6.5.1. Allowed Medications and Procedures

Topical, intra-articular, nasal, inhaled, and ophthalmic steroid therapies are not considered systemic and are allowed. Subjects may continue medications or treatments deemed necessary by the investigators to provide adequate supportive treatment for optimal medical care throughout the study, including previously prescribed medications for management of hypertension and lipid levels except for the prohibited medications listed in Section 6.5.2.

Subjects previously prescribed selective serotonin reuptake inhibitors or hormone replacement therapy are recommended to remain on a stable dose from at least 30 days prior to screening through the end of the study.

The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3 ~~([~~[e.g., ethyl esters of EPA or DHA]~~)~~, bile acid sequestrants, evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study.

The following **dosing windows** surrounding the infusion of CTX310 are suggested for lipid-lowering therapies:

- Monoclonal antibodies (e.g., alirocumab, evolocumab, and evinacumab) may not be administered beginning 7 days before the infusion of CTX310 and until 7 days after Day 1.
- Inclisiran may not be administered beginning 60 days before infusion of CTX310 and until 60 days after Day 1.
- Apheresis procedures may not be performed beginning 14 days before infusion of CTX310 and until 14 days after Day 1.

If a significant lowering of lipids (LDL-C or TG) is observed during the course of the study, i.e., lipid levels decrease to the desired levels (e.g., LDL-C <70 mg/dL [1.8 mmol/L], TG <150 mg/dL [1.7 mmol/L], or non–HDL-C <160 mg/dL [4.1 mmol/L]), adjustments to relevant medications or frequency of apheresis procedures may be instituted by the investigator after discussion with the sponsor (Section 4.1 Inclusion Criterion 7). It is expected that the plan for tapering other lipid-lowering medications or apheresis procedures will be individualized for each subject by the investigator depending on the response to study treatment, underlying genotype, and assessment of risk factors for future cardiovascular events.

6.5.2. Prohibited Medications

Medications prohibited prior to enrolling in the study or administration of CTX310 are noted in the exclusion criteria (Section 4.2) and are as follows:

- Hepatocyte-targeted small interfering RNA or antisense oligonucleotide molecules (except inclisiran).
- Any investigational product.
- Chronic systemic corticosteroids.
- Anabolic agents.
- Nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
- Prior treatment with a gene therapy/editing product.
- Vaccines should not be administered 30 days before or 30 days after CTX310 infusion.
- All oral anticoagulants (e.g., warfarin, apixaban, rivaroxaban, or dabigatran) should not be administered 30 days before or 30 days after CTX310 infusion.
- Phase 1b only: Evinacumab within 20 weeks prior to Day 1.

6.6. Lifestyle Considerations

Subjects who receive CTX310 must not donate eggs, sperm, blood, or organs for the duration of this study.

For subjects who consume alcohol the following recommendations are provided regarding alcohol intake:

- Abstain from alcohol for 2 weeks before and 2 weeks after CTX310 infusion.
- A maximum of 6 standard drinks per week but no more than 2 standard drinks per day for the duration of the study.

7. SAFETY MONITORING RULES

7.1. General Guidance

Subjects will be closely monitored for DLTs for 30 days after CTX310 infusion. Investigators are required to proactively monitor and treat all AEs in accordance with protocol guidance.

Although this is an FIH study and the clinical safety profile of CTX310 has not been previously described, the following general recommendations are provided based on prior experience with an LNP-formulated CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021) and *ANGPTL3*-targeted antisense oligonucleotide or interfering RNA therapies (Graham et al., 2017; Watts et al., 2022).

The safety profile of CTX310 will be continually assessed throughout the study, and investigators will be updated on a regular basis with new information regarding the identification and management of potential CTX310-related toxicity (refer to Investigator's Brochure).

7.1.1. Infusion-related Reactions

Infusion-related reactions have been reported with LNP utilizing treatments, occurring in up to 19% of patients receiving patisiran (Moghimi and Simberg, 2022). Infusion-related reactions can be either allergic reactions to foreign particles or non-immune mediated reactions. Most IRRs are mild and typically develop within minutes to several hours of initiation of the drug infusion, although symptoms may be delayed for up to 24 hours. IRRs may affect any organ system in the body. While most reactions are mild in severity, severe or fatal reactions can occur. The most common signs and symptoms of IRR are fever, chills, flushing, itching, alterations in heart rate (including tachycardia) or blood pressure (including hypotension), dyspnea, chest discomfort, back pain or abdominal pain, nausea, vomiting, diarrhea, and various types of skin rashes.

In the event of an acute IRR, the infusion of study drug will be slowed or stopped, and the subject closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study drug administration include, but are not limited to, acetaminophen/paracetamol, H1/H2 blockers, nonsteroidal anti-inflammatory drugs, epinephrine, supplemental oxygen, IV fluids, and/or corticosteroids. Caution should be exercised in the use of acetaminophen/paracetamol. Delayed IRR (>12 hours) are usually immune-mediated and respond best to corticosteroid treatment (e.g., methylprednisolone).

Following resolution of a mild or moderate IRR that required interruption or slowing of the study drug infusion, administration may resume or continue at the investigator's discretion at a slower infusion rate.

Study drug administration will not be resumed for any subject following a severe IRR until the case is discussed with the medical monitor.

7.1.2. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the Food and Drug Administration (FDA) 2009 draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation."

Any ALT or AST measurement that is >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) at any time during the study (treatment or post-treatment period), will be reported as AESI (Section 9.3) and the measurement(s) should be confirmed by repeat testing within 24 to 48 hours of all 4 of the usual serum measures (ALT, AST, alkaline phosphatase [AP], and total bilirubin) to confirm the abnormalities.

Subjects with confirmed ALT or AST levels >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) should have liver chemistry tests (ALT, AST, AP, INR, and total bilirubin) retested at least twice weekly until ALT and AST levels become ≤1.2 × ULN, or 1.2 × baseline value if the baseline value was >ULN. In addition, the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases.
2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
3. Obtain a history for travel and exposure to environmental chemical agents.
4. Serology for viral hepatitis (hepatitis A virus immunoglobulin M [IgM], hepatitis B surface antigen, hepatitis C virus antibody, cytomegalovirus IgM, and Epstein-Barr antibody panel).
5. Serology for autoimmune hepatitis (e.g., antinuclear antibody).

Additional liver evaluations, including gastroenterology/hepatology consultations, or hepatic computed tomography or magnetic resonance imaging (MRI), may be performed at the discretion of the investigator in consultation with the sponsor medical monitor. Repetition of the above evaluations should be considered if a subject's ALT and/or AST levels reach 5 × ULN.

8. STUDY PROCEDURES

A complete schedule of assessments is provided in Table 1. Descriptions of all required study procedures are provided in this section. In addition to protocol-mandated assessments, subjects should be followed per institutional guidelines, and unscheduled assessments should be performed when clinically indicated.

Missed evaluations should be rescheduled and performed as close to the originally scheduled date as possible except if rescheduling becomes, in the investigator's opinion, medically unnecessary or unsafe because it is too close in time to the next scheduled evaluation. In that case, the missed evaluation will be recorded as a protocol deviation and should be abandoned.

For the purposes of this protocol, there is no Day 0. All visit dates and windows are to be calculated using Day 1 as the date of CTX310 infusion.

8.1. Subject Screening, Enrollment, and Withdrawal

8.1.1. General Study Periods

8.1.1.1. Screening and Enrollment

Investigators will keep a log of all potential subjects reviewed and evaluated for study participation. Enrolled subjects are defined as subjects who consent to participate in the clinical study and whose eligibility is confirmed (meet the inclusion/exclusion criteria). Screen failures are defined as subjects who consent to participate in the clinical study but do not meet the eligibility criteria.

The screening period begins on the date that the subject signs the ICF and continues through confirmation of eligibility and enrollment into the study. Once informed consent has been obtained, the subjects will be screened to confirm study eligibility, as outlined in the schedule of assessments (Table 1). All screening assessments should be completed within 42 days after a subject signs the ICF. The medical monitor will review eligibility packets and verify information provided by the site to confirm agreement with the investigator that the subject is eligible for enrollment.

For the subject's convenience, if an assessment was performed before signing the ICF as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe.

Repetition of individual screening assessment(s) that did not meet eligibility requirements is not permitted with the following exceptions:

- If there is clear evidence of a laboratory error (e.g., hemolyzed sample) or equipment malfunction, collection of a repeat sample for the appropriate laboratory test or assessment may be permitted with the approval of the medical monitor.
- Individual laboratory results that, in the opinion of the investigator, are related to a temporary, reversible condition may be retested once after the condition resolves or within 7 days, whichever is earlier.

If repeat values of the individual assessment(s) are within the eligibility criteria and completed within the screening window, then the subject is eligible for the study.

Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

Specific procedures for enrollment will be provided to sites.

8.1.1.2. Infusion of CTX310

All subjects will receive a pretreatment regimen and study treatment, as described in Section 6.1.

8.1.1.3. Acute Safety Evaluation Period

The acute safety evaluation period is 30 days following infusion of CTX310 for each subject.

8.1.1.4. Follow-up and End of Study Definition

Following the 30-day acute safety evaluation period, subjects will be followed for an additional 11 months. Subjects will be considered to have completed the study after they complete the end of study (EOS) visit at Month 12.

The EOS is defined as the time at which the last subject completes the Month 12 visit, is considered lost to follow-up, withdraws consent, or dies.

8.1.1.5. Long-term Follow-up

To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310 and either discontinue prior to 12 months or complete this study will be asked to participate in a separate LTFU study for up to 15 years post infusion to assess long term safety, durability, and the occurrence of any clinical adverse event, including pancreatitis or cardiovascular events.

8.1.2. Subject Identification

A unique subject number will be assigned when an individual subject signs the study ICF. Subjects will be identified by a subject number consisting of:

> Last 3 digits of protocol number (400), assigned 3-digit site number, sequential 3-digit subject number (e.g., 400-XXX-YYY)

Once a number is assigned to a subject, it cannot be reassigned to a different subject. Rescreened subjects will keep the same subject number assigned during the initial screening process.

8.1.3. Replacement of Subjects

Subjects must receive CTX310 to be evaluated for a DLT. If a subject discontinues the study at any time prior to CTX310 infusion, the subject will be deemed unevaluable for DLT and will be replaced. If a DLT-evaluable subject (i.e., a subject who has been administered CTX310) has

signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared.

Subjects who discontinue from the study at any other time post–CTX310 infusion for any other reason will not be replaced.

8.1.4. Subject Withdrawal or Discontinuation

Subjects may voluntarily withdraw from the study at any time. Withdrawal of full consent means that the subject does not wish to receive further protocol-required therapy or undergo any study procedures. The sponsor will be notified of all study withdrawals. Subject data and samples collected up to the date of withdrawal of consent will be retained and included in the analyses. Where permitted by local regulations, publicly available data (e.g., death records) may be included after withdrawal of consent.

The investigator will specify reason for discontinuation from the study as follows:

- Subject withdrawal of consent.
- Investigator decision (only for subjects who did not receive study drug).
- Loss to follow-up.
- Death.

For subjects who are lost to follow-up (defined as 3 documented attempts to contact the subject via all available contact information in a reasonable time period), the investigator should attempt to search publicly available records (where permitted and allowed by local law) to ascertain vital status. For the duration of the study, attempts should also be made to collect information from other sources related to hospitalizations.

Subjects who withdraw completely from this study will be asked to participate in the separate long-term follow-up study if the long-term follow-up study has been approved at the investigative site. As CTX310 is not a continuously dosed investigational product, withdrawal from the study due to an AE is not applicable.

8.2. Study Assessments and Procedures

Refer to the schedule of assessments (Table 1) for the timing of the required procedures.

If needed, procedures may occur on separate days, but they must be done within the defined visit window.

8.2.1. Informed Consent

The investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, and possible risks and benefits of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated ICF must be obtained before conducting any study procedures (Section 13.4).

All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to rollover into a separate LTFU study for up to 15 years post-infusion (see Section 8.1.1.5 and Table 1).

Whenever important new information becomes available that may be relevant to the subject's consent, the written ICF and any other written information provided to subjects will be revised by the sponsor or designee and submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) for review. The agreed upon, revised ICF will be provided to each subject in the study for signing and dating. The investigator will explain the changes to the previous version.

8.2.2. Demographics and Medical History

Demographic data, including year of birth, sex, race, and ethnicity, will be collected. Medical history, including a full history of the subject's disease and response to treatment from date of diagnosis, will be obtained. Cardiac and surgical history will also be obtained.

Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment and during screening will be collected.

For study entry, all subjects must fulfill all inclusion criteria described in Section 4.1, and have none of the exclusion criteria described in Section 4.2.

8.2.3. Physical Exam, Height, and Weight

Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, Day 1, Day 30, and EOS visits, and the results documented.

Symptom-directed abbreviated physical examination may be performed at all other study visits. Changes noted from the examination performed at screening will be recorded as AEs.

Weight will be obtained according to the schedule of assessments (Table 1).

Height, BMI, and waist/hip ratio will only be obtained at screening and EOS.

8.2.4. Vital Signs

Vital signs will be recorded at every study visit according to the schedule of assessments (Table 1), and will include a single measurement of blood pressure, heart rate, respiratory rate, oxygen saturation by pulse oximetry, and temperature. On Day 1 and during hospitalization, measurements will be performed at the time points specified in the schedule of assessments (Table 1). Blood pressure and pulse rate should be measured using the site's equipment. The following guidance is recommended during taking vital signs.

1. Subject should be seated in a chair with their back supported, feet flat on the floor, and arms bared and supported at heart level.
2. The appropriate cuff size must be used to ensure accurate measurement and used consistently throughout the study.

3. Readings should be done on the same arm at each visit, preferably the non-dominant arm.
4. Measurement should begin after at least 5 minutes of rest.
5. Assessment of pulse rate can be manual or automated. When done manually, the rate should be counted in the brachial/radial artery for at least 30 seconds.

Other procedures should not be performed during blood pressure or heart rate measurements.

Vital sign measurements may be performed by the subject`s health care provider and reported to the investigator.

8.2.5. Liver Imaging

Standard local procedures will be used for image acquisition and analysis. A liver FibroScan or MRE (depending on availability) will be performed at screening and results will be used to exclude patients with liver stiffness consistent with signs of fibrosis (Section 4.2, Exclusion Criterion 2c). A 3-hour fast is recommended prior to FibroScan. A liver MRI–PDFF (for assessment of fatty liver/steatosis) will be performed at screening and EOS visits. Baseline liver fat status and quantitative change in hepatic steatosis will be collected within the case report form (CRF), with clinically significant findings reported as medical history or AEs, as appropriate.

8.2.6. Echocardiogram

A transthoracic echocardiogram is required at screening for assessment of LVEF for all subjects. (see Schedule of Assessment, Table 1). Additional echocardiograms may be obtained at the investigator's discretion.

8.2.7. Electrocardiogram

Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen. QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion.

8.2.8. Laboratory Tests

Laboratory samples will be collected and analyzed according to the schedule of assessments (Table 1). Unless stated, local laboratories meeting country-specific requirements for clinical testing will be utilized to analyze all tests. Laboratory assessments are listed in Table 4 and Table 5.

Table 4: Local Laboratory Testing

Serum chemistry	ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN/urea, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein
Cardiac biomarker	Troponin I or T
Urinalysis	Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal), ACR[1]
Coagulation	PT (INR), PTT/aPTT, fibrinogen
Serum or urine pregnancy test	hCG
CBC with differential	Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count, absolute lymphocyte count Note: The white blood cell count differential may be reported as absolute counts or as percentages, with the exception that absolute neutrophil and lymphocyte counts are required for eligibility assessment.
Thyroid function	T3, T4, TSH
Viral serology	HIV-1, HIV-2, HCV antibody or NAT, HBV surface antigen, HBV surface antibody, HBV core antibody or NAT

ACR: albumin to creatinine ratio; aPTT: activated partial thromboplastin time; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CBC: complete blood count; eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; HBV: hepatitis B virus; hCG: human chorionic gonadotropin; HCV: hepatitis C virus; HIV: human immunodeficiency virus; INR: international normalized ratio; MDRD: Modification of Diet in Renal Disease; NAT: nucleic acid testing; PT: prothrombin time; PTT: partial thromboplastin time; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone.
[1] Albuminuria (dipstick) or ACR to be performed at screening as specified in the schedule of assessments (Table 1).

Table 5: Central Testing

Genetic testing[1]	*LDLR*, *APOB*, *PCSK9, LPL*, *APOC2*, *APOA5*, *LMF-1*, *GPIHBP1*
Lipid panel	Total cholesterol, TG, HDL-C, non–HDL-C, LDL-C, VLDL-C, Lp(a), ApoB, ApoC-III [2, 3]

APOA5: apolipoprotein A5; ApoB or *APOB*: apolipoprotein B; *APOC2*: apolipoprotein C2; ApoC-III: apolipoprotein C-III; *GP1HBP1*: glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; *LDLR*: low-density lipoprotein receptor; *LMF-1*: lipase maturation factor 1; Lp(a): lipoprotein(a); LPL: lipoprotein lipase; *PCSK9*: proprotein convertase subtilisin/kexin type 9; TG: triglyceride(s); VLDL-C: very low-density lipoprotein cholesterol.
[1] Collection of a sample for genetic testing should not be repeated during rescreening, if applicable.
[2] Subjects on apheresis should have lipid levels sampled within 5 days prior to the procedure (a pre-apheresis sample on the day of apheresis is also adequate).
[3] All lipid panel samples testing to be performed after a minimum of an 8-hour fast.

8.2.9. Pregnancy Testing

All females must have pregnancy tests performed according to the schedule of assessments (Table 1). Serum pregnancy testing will be performed at screening. Urine or serum pregnancy test can be performed as per local standards at the EOS/M12 visit (Table 1, Table 4).

8.3. Immunogenicity

CTX310 is composed of mRNA encoding SpCas9 and [redacted] sgRNA targeting the gene of interest, encapsulated in an LNP. Blood samples will be collected as described in the schedule of assessments (Table 1) and stored for potential future immunogenicity assessments (anti-drug antibody [ADA] to LNP and Cas9), if required.

8.4. Pharmacokinetics and Pharmacodynamics Assessments

8.4.1. CTX310 Pharmacokinetic Analysis

PK analysis of [redacted](LNP), [redacted](LNP), and Cas9 protein levels will be performed on blood samples collected per the schedule of assessments (Table 1). On Day 1, samples will be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at the scheduled time points after the completion of CTX310 infusion, as described in the schedule of assessments (Table 1). For all other time points specified in the schedule of assessments (Table 1), including D2, D3, D4, a single sample will be collected as described.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.2. ANGPTL3

Plasma samples will be obtained to follow the ANGPTL3 concentration, as described in the schedule of assessments (Table 1).

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3. Exploratory Biomarker Research

Exploratory biomarker research may be conducted to identify genomic, metabolic, and/or proteomic biomarkers that may be indicative or predictive of clinical response, resistance, safety, PD activity, and/or the mechanism of action of treatment. In addition, samples collected for protocol-specific endpoints will be used for exploratory research, pending availability of excess sample.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3.1. Whole Blood

Whole blood samples will be obtained and stored at screening.

8.4.3.2. Plasma

Plasma samples for storage will be obtained at screening.

8.4.3.3. Serum

Serum samples will be obtained to follow exploratory biomarkers (e.g., cytokines), as described in the schedule of assessments (Table 1).

9. SAFETY, ADVERSE EVENTS, AND STUDY OVERSIGHT

The investigator will monitor each subject for clinical and laboratory evidence of AEs on a routine basis throughout the study and assess and record details as described in Section 9.1. AEs in response to a query, observed by site personnel, or reported spontaneously by the subject will be recorded. The investigator is responsible for ensuring that any AEs observed by the investigator or reported by the subject are recorded in the subject's medical record in the sponsor's electronic data capture system. AEs will be followed through to event resolution or stability or death.

9.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not considered related to the medicinal (investigational) product [Guidelines for Good Clinical Practice (GCP) E6(R2)]. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

The investigator will assess and record information pertaining to the AE, which includes but is not limited to the following: date of onset, event diagnosis (when known) and/or signs and symptoms, duration, severity, seriousness, relationship to the study therapy or procedure, action(s) taken, and outcome.

Additional criteria defining an AE are described below.

The following **are** considered AEs:

- Aggravation of a pre-existing disease or permanent disorder (any clinically significant worsening in the nature, severity, frequency, or duration of a pre-existing condition).
- Events resulting from protocol-mandated procedures (e.g., complications from invasive procedures).

The following **are not** considered AEs:

- Elective or preplanned medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. These should be recorded in the relevant eCRF.

  Note: An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or SAE.
- Pre-existing diseases or conditions that do not worsen during or after administration of the investigational medicinal product.
- Hospitalization planned for study treatment infusion or observation.

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a clinically significant change from the subject's baseline value. Only abnormal laboratory results considered by the investigator to be clinically significant should be reported as AEs (e.g., an abnormal laboratory finding associated with clinical symptoms, of prolonged duration, or that requires additional monitoring and/or medical intervention). Whenever possible, these should be reported as a clinical diagnosis rather than the abnormal parameter itself (i.e., neutropenia vs neutrophil count decreased). Abnormal laboratory results without clinical significance should not be recorded as AEs.

AEs can occur before, during, or after treatment, and can be either treatment-emergent (AEs that start or worsen on or after CTX310 infusion) or non–treatment-emergent. A non–treatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that occurs after written informed consent has been obtained and before the subject has received CTX310.

9.2. Serious Adverse Event

An AE of any untoward medical consequence must be classified as an SAE if it meets any of the following criteria:

- Results in death.
- Is life-threatening (i.e., an AE that, in the opinion of the investigator, places the subject at immediate risk of death).
- Requires inpatient hospitalization or prolongs an existing hospitalization (hospitalizations for scheduled medical or surgical procedures or to conduct scheduled treatments do not meet these criteria).
- Results in persistent or significant disability or incapacity.
- Results in a congenital anomaly or birth defect in a newborn.
- Other important/significant medical events. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Hospitalization for study treatment infusions or planned hospitalizations following CTX310 infusion are not considered SAEs. Furthermore, hospitalizations for observation or prolongation of hospitalization for observation alone should not be reported as an SAE unless they are associated with a medically significant event that meets other SAE criteria, as assessed by the investigator.

9.3. Adverse Events of Special Interest

An AESI, whether serious or nonserious, is one of scientific and medical concern specific to the sponsor's product for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate.

The following events and/or laboratory findings will be designated as AESIs based on the predicted pharmacology, nonclinical safety profile, possible off-target effects, and/or adverse reactions seen in studies of other ANGPTL3 inhibitors (see Investigator's Brochure for details):

- IRRs.
- Abnormal coagulation findings, defined as clinically relevant abnormal bleeding, thrombotic, or hemorrhagic events.
- Increase in ALT or AST: ≥3 × ULN or ≥2 × the baseline value (if baseline ALT ≥ULN).
- Allergic reactions/events or localized reactions (collected for 30 days post-infusion).
- New malignancy.

Additional information on the required AESI reporting collection period is detailed in Table 7.

9.4. Adverse Event Severity

AESIs and DLTs will be graded using CTCAE version 5.0. If CTCAE v5.0 grade or protocol-specified criteria are not applicable, AE toxicity should be graded according to Table 6.

Table 6: Adverse Event Severity

Severity Grade	Description
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL. [1]
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. [2]
4	Life-threatening consequences: urgent intervention indicated.
5	Death related to AE.

ADL: activities of daily living; AE: adverse event.
[1] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[2] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

9.5. Adverse Event Causality

The investigator must assess the relationship between each AE and CTX310 and any protocol-mandated study procedure (all assessed individually). The assessment of relationship will be made based on the following definitions:

- **Related**: There is a clear causal relationship between the study treatment or procedure and the AE.
- **Not related**: There is no evidence to suggest a causal relationship between the study treatment or procedure and the AE.

Investigators should consider the temporal association between the timing of the event and administration of the treatment or procedure, a plausible biological mechanism, and other potential causes of the event (e.g., concomitant therapy, underlying disease) when making their assessment of causality.

If an AE is assessed to be not related to any study intervention, an alternative etiology must be provided in the CRF.

9.6. Outcome

The outcome of an AE will be classified and reported as follows:

- Fatal.
- Not recovered/not resolved.
- Recovered/resolved.
- Recovered/resolved with sequelae.
- Recovering/resolving.
- Unknown.

9.7. Adverse Event Collection Period

The safety-related information of all subjects in this study will be recorded from the time of ICF signing until EOS/M12; however, there are different reporting requirements for the different time periods in the study. Table 7 describes the AEs that should be reported at each time period of the study.

Table 7: Adverse Event Collection by Study Time Period

Time Period	AE Reporting Requirements
Informed consent to 30 days after CTX310 infusion	All AEs
30 days after CTX310 infusion through Month 12 visit	• Nonserious AEs related to study procedure[1] or CTX310 • SAEs • AESIs

AE: adverse event; AESI: adverse event of special interest; SAE: serious adverse event.
[1] AEs related to study procedures include events related to, e.g., laboratory procedures, imaging, or prophylaxis regimen, and may occur at any time starting at screening and continuing through the duration of the study.

If a subject does not receive CTX310 therapy, the AE reporting period ends.

9.8. Adverse Event Reporting

All AEs will be recorded in the appropriate section of the eCRF. Subjects withdrawn from the study because of AEs will be followed by the investigator until the outcome is determined. When appropriate, additional written reports and documentation will be provided.

AE reporting should occur as per the study period designated in Table 7. If a reportable SAE or AESI occurs, the SAE/AESI form provided to investigators should be completed and submitted to the sponsor or its designee immediately (i.e., no more than 24 hours after the investigator becomes aware of the event) by scanning and emailing the paper report to:

globalpv@crisprtx.com (for notifications or questions)

In particular, if the SAE is fatal or life-threatening, the report form must be submitted immediately, irrespective of the extent of available AE information. The timeframe also applies to additional, new information (follow-up) on previously reported SAE/AESI reports.

In the rare event that the investigator does not become aware of the occurrence of a reportable SAE/AESI immediately (e.g., a study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours of learning of the event and document the date and time of awareness of the event.

In addition, an investigator may be requested to obtain specific additional follow-up information in an expedited fashion (e.g., autopsy finding). The information collected for SAEs/AESIs is more detailed than that captured on the AE CRF. In general, this will include a description of the event in sufficient detail, as well as concomitant medications and any relevant medical details to allow for a complete medical assessment of the case and causality assessment by the investigator and the sponsor. Information on the other possible causes of the event, such as concomitant medications and illnesses must be provided.

The investigator must complete, sign, and date the SAE/AESI form, verify the accuracy of the information recorded on the SAE/AESI form with the corresponding source documents, and send

a copy by email or fax to the sponsor or designee. Subsequently, all SAEs/AESIs will be reported to the health authorities per local reporting guidelines.

It is the principal investigator's responsibility to notify the IRB or EC of all SAEs that occur at his or her site as per local policies. Investigators will also be notified of all unexpected, serious, drug-related events that occur during the clinical study. Each site is responsible for notifying its IRB or EC of these additional SAEs.

9.9. Medication Errors and Overdose

An overdose or medication error along with any associated AE, regardless of seriousness, should be reported to the sponsor on the provided SAE/AESI form within 24 hours of awareness of the medication error/overdose or associated AE.

9.10. Pregnancy

Certain information, although not considered an AE or SAE, must be recorded, reported, and followed as indicated for an SAE (see Section 9.8), including pregnancies.

Pregnancies (both those of female subjects and female partners of male subjects) must be reported to the sponsor or designee within 24 hours of the investigator's knowledge using the Investigational Product Pregnancy Report. All pregnancies will be followed through outcome and the infant will be followed at birth and at 1 year, provided informed consent is obtained. Pregnancy outcome must be reported to the sponsor or designee using the pregnancy outcome section of Investigational Product Pregnancy Report.

Pregnancies themselves are not considered AEs or SAEs. However, any AEs or SAEs occurring during pregnancy are to be reported following AE and SAE reporting guidelines. Any congenital anomalies and birth defects in the infant should be reported as an SAE. Additionally, any CTX310-related malignancy or death in the infant during the 12-month follow-up period should also be reported as an SAE.

9.11. Reporting Deaths

Regardless of relationship to the investigational product, all deaths on study should be recorded in the relevant eCRF. Additionally, all AEs with an outcome of death, regardless of relationship to investigational product, should be reported to the sponsor as SAEs, to Pharmacovigilance on the provided SAE/AESI form.

9.12. Study Oversight

9.12.1. Safety Review Committee

During dose escalation, an SRC consisting of investigators and sponsor representatives will review all available safety data and make decisions regarding dose escalation or de-escalation. During dose escalation, the SRC may also propose revisions to the DLT definitions and dosing schema and will continue to meet regularly to discuss toxicity management algorithms and review individual subject cases. Following discussion with the SRC, the sponsor may consult

with the independent DSMB regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema.

During follow-up, the SRC may continue to meet on an ad hoc basis to discuss 1 or more of the following: (1) single-subject case studies, (2) aggregate safety and/or biomarker data, (3) toxicity management algorithms, and (4) review and discuss the possibility of expanding the study to include other study populations.

The SRC may be consulted on other aspects of the study conduct, as applicable.

9.12.2. Independent Data Safety Monitoring Board

An independent DSMB comprised of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise to monitor the study will be established during dose escalation. Throughout the study the DSMB will review safety data from dose escalation and dose expansion. The DSMB will review safety data pertaining to stopping rules provided by the sponsor, as detailed in the DSMB charter. The DSMB may recommend that the sponsor amend the protocol, stop enrollment, or discontinue the study at any time if concerns about safety of the subjects are encountered. The roles and responsibilities of the DSMB will be further described in the DSMB charter.

10. STOPPING RULES AND STUDY TERMINATION

10.1. Stopping Rules for the Study

Administration of the investigational product will be paused temporarily for safety reasons, pending review of the data by the sponsor, SRC, and DSMB upon the occurrence of laboratory results meeting any of the criteria in Section 10.1.1. Laboratory values that are not confirmed due failure to retest or missing laboratory values will be presumed confirmed.

Subjects who have already received a dose of study drug will continue with follow-up as outlined in the protocol. No further dosing of subjects will occur until an assessment of the data is completed by the sponsor, the SRC, and the DSMB. Notification of the suspension of accrual will be provided to the health authorities and ECs. If deemed appropriate based on the sponsor, the SRC, and the DSMB review, a rationale with data supporting continued dosing without a change to the protocol will be submitted to the health authorities and ECs for review and approval. If a modification is required, a protocol amendment will be submitted.

10.1.1. Stopping Rules

Treatment of the next subject will be paused as described in Section 10.1 if any of the following criteria are met:

1. ≥2 subjects at a DL with ALT or AST >8 × ULN, which is confirmed and persists for >14 days.
2. ≥2 subjects at a DL with ALT or AST >5 × ULN, which is confirmed and persists for >30 days.
3. ≥2 subjects at a DL with ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5 × ULN persisting for >14 days.
4. Thrombosis, hemorrhage, and/or laboratory parameters consistent with disseminated intravascular coagulation or bleeding that are likely related to study drug.
5. Fatal or life-threatening AE that is due to investigational product.
6. Any other AE that poses an immediate hazard to study subjects, in the opinion of the sponsor in consultation with the investigators and SRC.

10.2. Stopping Rules for Individual Subjects

Stopping rules for individual subjects are as follows:

- Any medical condition that, in the opinion of the investigator or sponsor, would put the subject at risk during continuing study-related treatments or follow-up.
- If a subject is found to have not met all the eligibility criteria or has a major protocol deviation before the start of CTX310 infusion and in the opinion of the investigator.
- Would put the subject at risk for continuing study-related procedures or follow-up.

10.3. Study Termination

This study may be discontinued at any time due to safety concerns, failure to meet expected enrollment goals, administrative reasons, or at the discretion of the sponsor. In the event of study termination, the sponsor will immediately inform all appropriate parties, including principal investigators, ECs, IRBs, and competent authorities. In the event this study is terminated early, subjects who have received CTX310 will be asked to participate in a separate LTFU study for up to 15 years post-infusion.

11. STATISTICAL ANALYSES

11.1. Study Objectives and Hypotheses

The primary objective is to evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or LDL-C and/or ApoB, and to determine the RP2D.

The secondary objectives are to further characterize the safety and tolerability, and to assess the preliminary efficacy, PK, and PD of CTX310. No formal hypothesis testing will be performed.

11.2. Study Endpoints

11.2.1. Primary Endpoints

- Incidence of DLTs

11.2.2. Secondary Endpoints

11.2.2.1. Secondary Efficacy Endpoints

- Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline.

11.2.2.2. Secondary Safety Endpoints

- Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs

11.2.2.3. Secondary Pharmacokinetics/Pharmacodynamics Endpoints

- Assess the plasma levels of LNP ([redacted] and [redacted]).
- Assess the plasma level of Cas9 protein.
- Percentage change in ANGPTL3 concentration over time compared to baseline.

11.2.3. Exploratory Endpoints

- Percentage change in free fatty acid (FFA) levels over time compared to baseline.
- Change in fatty liver disease.
- Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required).

For Phase 1b subjects only

- Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG

11.3. Analysis Sets

The following analysis sets will be evaluated and used for presentation of the data.

- The enrolled set includes all subjects who sign the informed consent and meet the inclusion/exclusion criteria.
- The safety analysis set is a subset of the enrolled set that includes subjects who receive the CTX310 infusion. Analyses of the safety assessments will be based on the safety analysis set. Subjects in the safety analysis set will be classified by received CTX310 DL.
- The DLT evaluable set includes all subjects during dose escalation who receive CTX310 infusion and complete the DLT evaluation period or discontinue early after experiencing a DLT. The DLT period will begin with CTX310 infusion and last for 30 days, or beyond for improvement or resolution of the signs or symptoms of a potential DLT. The DLT evaluable set will be used for dose escalation decisions.
- The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL.

11.4. Sample Size

The sample size of the study will be approximately 69 subjects. This will include approximately 21 subjects in Phase 1a dose escalation and approximately 48 subjects in the Phase 1b disease-specific cohort expansion parts of this study. In Phase 1b, there will be up to 12 subjects per cohort.

No formal sample size calculation was performed for this study. The total sample size is estimated based on the dose escalation/expansion design and considered sufficient for the preliminary evaluation of the safety and tolerability of CTX310.

11.5. Interim Analysis

No formal efficacy interim analysis is planned.

The DSMB will review safety and efficacy data as needed during the study to monitor stopping rules and to provide recommendations on enrollment or protocol amendment.

11.6. Planned Method of Analyses

The primary analysis will occur after all subjects have completed 26 weeks of follow-up after CTX310 infusion or discontinued earlier. A final analysis will occur when all subjects complete or withdraw from the study. Tabulations will be produced for appropriate disposition, demographic, baseline, efficacy, and safety parameters. By-subject listings will be provided for all data, unless otherwise specified.

11.6.1. Efficacy Analysis

The FAS will be used as the analysis set for efficacy. The efficacy endpoints of percentage change in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each DL. Categorical summaries at selected time points, including 26 and 52 weeks, based on appropriate cutoff may be provided.

11.6.2. Safety Analysis

Safety analysis will be conducted on the safety analysis set. Summaries of DLTs and other AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each DL of CTX310 and overall.

Summaries of AEs will focus on TEAEs, defined as AEs that start or worsen on or after CTX310 infusion. AEs will be graded according to CTCAE v5.0. The incidence of TEAEs will be summarized by system organ class and preferred term, grade, and relation to CTX310. Key subsets of TEAEs, including DLTs, AESIs, grade ≥3 AEs, related AEs, and SAEs, will be summarized separately.

Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of clinically significant laboratory abnormalities and clinically significant abnormal vital signs will be summarized.

The incidence of other clinically significant safety measure abnormalities (e.g., ECG) will also be summarized, if applicable.

11.6.3. Pharmacokinetic and Pharmacodynamic Analyses

Plasma levels of LNPs ([redacted] and [redacted]) and Cas9 protein over time will be summarized using descriptive statistics. Exploratory analysis based on an applicable PK model may be performed.

Percentage change in ANGPTL3 concentration over time compared to baseline will be summarized using descriptive statistics.

11.6.4. Biomarker Analyses

Additional exploratory biomarkers, including FFA levels, fatty liver disease marker(s), and immunogenicity marker(s), if data are available, will be summarized using descriptive statistics.

11.6.5. Exploratory Analysis of Pancreatitis Event

The annualized event rate of pancreatitis after CTX310 will be estimated using Poisson model and compared to baseline to assess the effect of CTX310 on pancreatitis event.

11.6.6. Patient-reported Outcome Analyses

Not applicable.

12. DATA MANAGEMENT

12.1. Data Recording and eCRF Processing

The investigator is required to maintain adequate and accurate medical records designed to record all observations and data pertinent to the study for each subject. Study data for each consented subject will be entered into a CRF by site personnel using a secure, validated (Part 11 of Title 21 of the Code of Federal Regulations–compliant), web-based electronic data capture application. Instances of missing, discrepant, or uninterpretable data will be queried by the sponsor or designee for resolution. Any changes to study data will be made to the eCRF and documented in an audit trail maintained within the clinical database. CRFs must be reviewed and electronically signed and dated by the investigator.

An audit may be performed at any time during or after completion of the clinical study by sponsor personnel or their designee. All study-related documentation must be made available to the designated auditor.

13. ADMINISTRATIVE

13.1. Institutional Review Board/Ethics Committee

This protocol and the proposed ICF must be reviewed and approved by the appropriate IRB/EC prior to the start of the study. During the study, the investigator shall make timely and accurate reports to the IRB/EC on the progress of the study at intervals not exceeding 1 year, as well as satisfying any other local IRB/EC regulations regarding reporting. Copies of all reports to and correspondence with and from the IRB/EC must be provided to the sponsor or its designee.

Any significant changes or revisions in the study protocol or any changes that may alter subject risk must be approved in writing by the IRB/EC prior to implementation. A protocol change intended to eliminate an apparent imminent hazard may be implemented immediately provided that the sponsor is promptly notified, and an amendment is subsequently provided by the sponsor and approved by the IRB/EC.

It is the investigator's obligation to maintain an IRB/EC correspondence file, and to make this available for review by sponsor representatives or their designee as part of the study monitoring process.

13.2. Study Conduct

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Guidelines for GCP and applicable regulatory requirements.

13.3. Subject Privacy

To maintain subject confidentiality and to comply with applicable data protection and privacy laws and regulations, all data provided to the sponsor or designee, study reports, and communications relating to the study will identify subjects by assigned subject numbers, and access to subject names linked to such numbers shall be limited to the site and the study investigator and shall not be disclosed to the sponsor or designee. As required by applicable laws and regulations in the countries in which the study is being conducted, the investigator will allow the sponsor and/or its representatives access to all pertinent medical records to allow for the verification of data gathered and the review of the data collection process. The regulatory authorities in other jurisdictions, including the IRB/EC, may also request access to all study records, including source documentation, for inspection.

13.4. Written Informed Consent

The investigator will be responsible for obtaining written informed consent from potential subjects prior to any study-specific screening and entry into the study. The source documents for each subject shall document that the informed consent was obtained prior to participation in the study.

The investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, and possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject

should be given the opportunity to ask questions and allowed time to consider the information provided. The investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the subject. Whenever important new information becomes available that may be relevant to the subject's consent, the written ICF and any other written information provided to subjects will be revised by the sponsor or designee and be submitted to the IRB/EC for review and favorable opinion. The agreed upon, revised information will be provided to each subject in the study for signing and dating. The investigator will explain the changes to the previous version.

13.5. Delegation of Investigator Responsibilities

The investigator will ensure that all persons involved in the conduct of the study are informed about the protocol, protocol amendments, study procedures, and study-related duties.

13.6. Study Files

Documentation concerning investigators' credentials and experience, and IRB approval of protocol and ICF, and other documentation are required prior to shipment of the investigational product to the study site. Copies of these documents as well as supplemental information, such as the Investigator's Brochure, will be kept onsite in an investigator study file binder. This file also will contain investigational product accountability (receipt/dispensing) records, sponsor/investigator correspondence, IRB correspondence, changes to the protocol, information regarding monitoring activities, subject exclusion records, and biological sample records.

13.7. Retention of Study Documents

All study documents, including records of investigational product receipt and disposition, copies of eCRFs, as well as supporting documentation and administrative records, must be retained by the investigator for a minimum of 2 years following notification that the appropriate regulatory authority has approved the product for the indication under study, notification that the entire clinical investigation will not be used in support of a marketing application, or notification that the marketing application was not approved. No study documents will be destroyed or moved to a new location without prior written approval from the sponsor. If the investigator relocates, retires, withdraws from the clinical study for any reason, or dies, all records required to be maintained for the study should be transferred to an agreed upon designee, such as the study monitor, another investigator, or the institution where the study was conducted. The sponsor should be notified in writing at least 30 days prior to the disposal of any study records related to this protocol.

13.8. Protocol Compliance

No modifications to the protocol will be made without the approval of both the investigator and the sponsor. Changes that significantly affect the safety of the subjects, the scope of the investigation, or the scientific quality of the study (e.g., efficacy assessments) will require IRB/EC notification before implementation, except where the modification is necessary to eliminate an apparent immediate hazard to human subjects. The sponsor will submit all protocol modifications to the required regulatory authorities.

Emergency departures from the protocol that eliminate an apparent immediate hazard to a particular subject and that are deemed crucial for the safety and well-being of that subject may be instituted for that subject only. The investigator or other attending physician also will contact the sponsor as soon as possible in the case of such a departure. These departures do not require preapproval by the IRB; however, the IRB and sponsor must be notified in writing as soon as possible after the departure has been made. In addition, the investigator will document the reasons for protocol deviation and the ensuing events.

13.9. Monitoring Functions and Responsibility

Before an investigational site can enter a subject into the study, a sponsor representative will evaluate the investigational study site to:

- Determine the adequacy of the facilities.
- Discuss with the investigator(s) and other personnel their responsibilities regarding protocol adherence, and the responsibilities of the sponsor or its representatives. This will be documented in a Clinical Study Agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or representative will have regular contacts with the investigational site to:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, and that investigational product accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each subject (e.g., clinic charts).
- Record and report any protocol deviations not previously sent to the sponsor.
- Confirm AEs and SAEs have been properly documented on CRFs, that any SAEs and AESIs have been forwarded to the sponsor, and SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

13.10. Quality Control and Quality Assurance

Authorized representatives of the sponsor, a regulatory authority, an EC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded,

analyzed, and accurately reported per the protocol, ICH/GCP guidelines, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

13.11. Disclosure of Data

All information obtained during the conduct of this study will be regarded as confidential. Disclosures (i.e., any release of information to any third party not noted herein) of any information not previously known to be public and/or results of the investigation for publication or by capsules or poster presentation shall not be made earlier than 30 days after submission of the proposed material to the sponsor for inspection, unless the sponsor consents to earlier disclosure. The investigator will take appropriate cognizance of the sponsor's suggestions before disclosure for publication or presentation consistent with protection of the sponsor's right to its confidential data.

13.12. Confidentiality and Publication

All scientific, commercial, and technical information disclosed by the sponsor in this protocol or elsewhere will be considered the confidential and proprietary property of the sponsor. The investigator will hold such information in confidence and shall not disclose the information to any third party except to such of the investigator's employees and staff as have been made aware that the information is confidential and who are bound to treat it as such and to whom disclosure is necessary to evaluate that information. The investigator will not use such information for any purpose other than determining mutual interest in performing the study and, if the parties decide to proceed with the study, for the purpose of conducting the study.

The investigator understands that the information developed from this clinical study will be used by the sponsor in connection with the development of the investigational product and other drugs and diagnostics, and therefore may be disclosed as required to other clinical investigators, business partners and associates, the FDA, and other government agencies. The investigator also understands that to allow for the use of the information derived from the clinical study, the investigator has the obligation to provide the sponsor with complete test results and all data developed in the study.

Authorship of publications will be determined based on the Recommendations for Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, which states that authorship should be based on the following 4 criteria:

1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;
2. Drafting of the article or revising it critically for important intellectual content;
3. Final approval of the version to be published; and
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

An individual must meet all criteria to be an author on any publication containing data from this study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between the sponsor and the investigator and/or the investigator's institution.

13.13. Clinical Study Report

After completion of the study, a clinical study report, written by the sponsor or designee in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

14. APPENDIX: DEFINITIONS AND SUPPORTING OPERATIONAL DETAILS

14.1. Lean Body Weight Calculation

The eLBW will be estimated using the equation developed and validated by (Janmahasatian et al., 2005).

Females: LBW[kg] = (9270 × Total_body_weight[kg]) / (8780 + (244 × BMI[kg/m^2]))

Males: LBW[kg] = (9270 × Total_body_weight[kg]) / (6680 + (216 × BMI[kg/m^2]))

Where BMI[kg/m^2] = Total_body_weight[kg] / Height[m]^2

14.2. Contraception Requirements

Females of childbearing potential are excluded from the study.

Female Subjects of Nonchildbearing Potential

Female subjects of nonchildbearing potential will not be required to use contraception. To be considered of nonchildbearing potential, female subjects must meet at least 1 of the following criteria:

- Postmenopausal: At least 12 consecutive months of amenorrhea in women with a uterus without an alternative medical cause; **or**
- Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).

Note: All other female subjects (including subjects with tubal ligations and subjects who do not have a documented hysterectomy) will be considered of childbearing potential.

Acceptable methods of contraception for male subjects and their partners are listed below. If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.

Male Subjects

Acceptable contraceptive methods must be used from study informed consent through 12 months after CTX310 infusion and include the following:

- Nonsterile male subjects who are or may become sexually active with female partners of childbearing potential must agree to use an acceptable, effective method of contraception from study informed consent through 12 months after CTX310 infusion.
- If the male is infertile (e.g., bilateral orchiectomy). Infertility may be documented through examination of a semen specimen or by demonstration of the absence of the vas deferens by ultrasound.

- True abstinence for the subject, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.
- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise, condom and spermicide as separate products). Local regulations may require use of an additional acceptable method of contraception. Note: Male condoms without spermicide may be used with another acceptable method of female contraception listed below (see acceptable contraceptive methods for female partners of male subjects).
- Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of enrollment, and 1 barrier method of contraception.

Acceptable Contraceptive Methods for Female Partners of Male Subjects:

- Bilateral tubal ligation performed at least 6 months previously.
- Continuous use of an intrauterine device for at least 90 days before consent.
- Hormonal contraceptives, if successfully used for at least 60 days before consent.

Additional Notes:

- Female condom cannot be used with male condom (as a double method of contraception) due to risk of tearing.
- The use of birth control methods does not apply if the female partner has had a bilateral oophorectomy, hysterectomy, or is postmenopausal.
- Male subjects who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.
- If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.
- Male subjects must not donate sperm throughout the study, and for 12 months following CTX310 infusion.
- Unique situations that may not fall within the above specifications may be discussed with the sponsor medical monitor or designee on an individual basis.

14.3. Country/Region-Specific Differences

Not applicable for this version of the protocol.

14.4. Protocol Amendments

The summary of key changes for the current amendment is located above the Table of Contents. The current protocol is Version 6.0, Amendment 5.

14.4.1. Summary of Changes to the Current Protocol V6.0, Amendment 5 (Global)

<table>
<tr><th>Change</th><th>Rationale</th><th>Affected Section(s)</th></tr>
<tr><td colspan="3">Changes specific to Section 1.1 Protocol Synopsis (identical changes in the synopsis and the body of the protocol, Sections 2 through 14, are captured in chronological order by protocol body section level heading)</td></tr>
<tr><td>Title Page - Administrative Update
Original:
<table><tr><td>Sponsor Emergency Contact</td><td>~~Sandeep Soni, MD~~
~~Executive Director,~~
~~Clinical Development~~
~~CRISPR Therapeutics~~
~~455 Mission Bay Blvd South~~
~~3rd Floor~~
~~San Francisco, CA 94158~~ USA</td><td>+1 ~~650 448 8044~~</td></tr></table>Modified to:<table><tr><td>Sponsor Emergency Contact</td><td>Jason Duran, MD, PhD
Head of Cardiovascular
Clinical Development
CRISPR Therapeutics
105 W First St
Boston, MA 02127 USA</td><td>+1 617-315-4600</td></tr></table></td><td>Administrative update</td><td>Title Page</td></tr>
<tr><td colspan="3">Section 1.1 Protocol Synopsis</td></tr>
<tr><td>Study Population – addition of text
Modified to: “The Phase 1a dose escalation will include eligible subjects, regardless of underlying dyslipidemia subtype. The majority of subjects enrolled in Phase 1a are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia and HTG. Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk.
The Phase 1b cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD, that encompass HTG and/or hypercholesterolemia in the following disease-specific cohorts:
Severe HTG (>500 mg/dL triglycerides)
HoFH
HeFH
Mixed hyperlipidemias (including moderate HTG)</td><td>Added text to describe Phase 1b</td><td>Section 1.1 Protocol Synopsis</td></tr>
</table>

Change	Rationale	Affected Section(s)
Study Design – added text Modified to: "*When the DLT evaluation period ends for the last subject enrolled at each escalation DL, the Safety Review Committee (SRC) will review pharmacokinetic (PK)/pharmacodynamic (PD) and safety data and will be responsible for making decisions regarding dose escalation or de-escalation.* *Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D). The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.* *Phase 1b – Disease-specific Cohort Expansion* *Following completion of eLBW-based dose escalation, the sponsor will review the totality of safety and clinical activity data to delineate a flat dose (FD) for Phase 1b or anticipated RP2D. The FD will be applied to the Phase1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. An additional FD may be evaluated in each expansion cohort if the safety or activity profile of the first FD is inadequate but and will not exceed the equivalent highest dose evaluated during Phase 1a. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase 1a. Phase 1b will confirm the RP2D.* *The SRC will be responsible for endorsing the recommended phase to dose (RP2D).*"	Added text describe the process for data review in Phase 1a and Phase 1b	Section 1.1 Protocol Synopsis
Study Oversight *Safety Review Committee* *Modified to: "Following completion of dose escalation and analysis of PK/PD, key lipid, and safety data in Phase 1a, the SRC will evaluate the totality of clinical data to approve a FD for evaluation in Phase 1b expansion cohorts."* *Independent* Data Safety Monitoring Board Throughout the study the DSMB will review safety from dose escalation *and disease specific cohort expansions*.	Added text to describe the responsibilities of the SRC and DSMB in Phase 1a and Phase 1b	Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Proposed Starting Dose and Dose Escalation ***Phase 1b – Disease-specific Cohort Expansion*** *Following completion of eLBW-based dose escalation (Table S1), analysis of PK/PD, key lipid and safety data, and approval by the SRC, a safe and efficacious FD will be evaluated in 4 disease-specific cohort expansion cohorts. Each disease-specific cohort may enroll up to 12 subjects.* *Following endorsement from the SRC, the sponsor will declare the RP2D based on the totality of clinical data for each disease-specific cohort for future studies. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 during dose escalation may be eligible for a second dose based on predefined criteria provided in a future amendment or separate study protocol.* *Toxicities will be graded and documented according to the criteria described in the protocol.* *All cumulative AEs occurring outside the DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the DSMB.*	Added 2 dose levels to Phase 1a dose escalation and added descriptive text regarding dosing of CTX310 in Phase 1b	Section 1.1 Protocol Synopsis
Section 1.2 Study Schema		
Section 1.2 Study Schema – addition of text specifying Phase 1a in Footnote b and Acute Safety Assessment Modified to: " ≥14-day stagger between each subject within a DL *in Phase 1a dose escalation only*. Footnote b: For each DL *during Phase 1 a dose escalation*, there will be a safety monitoring period for ≥14 days between the treatment of each subject an any subsequent subjects within the DL….."	Clarification that dose stagger applies to Phase 1a only	Section 1.2 Study Schema
Section 1.3 Schedule of Assessments, Table 1 Schedule of Assessments		
Section 1.3 Schedule of Assessments Table 1 Schedule of Assessment – new row and new footnote New row: "*History of pancreatitis at Screening (Day -42 to -1)"* Modified to: "*4. Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment will be collected. Section 8.2.2."* Subsequent footnotes renumbered.	Added table row and footnote to describe medical history requirement for subjects in Phase 1b with severe HTG	Section 1.3 Schedule of Assessments
Section 1.3 Schedule of Assessments and Section 8.1.1.1 Screening and Enrollment Revision of table footnote #1: Modified to: "*For the subject's convenience, if an assessment was performed before signing the Informed Consent Form as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe."*	Clarification of assessment to avoid unnecessary procedures.	Section 1.3 Schedule of Assessments and Section 8.1.1.1 Screening and Enrollment

Change	Rationale	Affected Section(s)
Section 2 Introduction		
Section 2.1 Dyslipidemias – deleted redundant text Original: Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on the ~~following~~ monogenic or polygenic refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes: • ~~Multifactorial chylomicronemia syndrome (MCS).~~ • ~~Homozygous familial hypercholesterolemia (HoFH).~~ • ~~Heterozygous familial hypercholesterolemia (HeFH).~~ • ~~Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).~~ • ~~Other HTG and/or hypercholesterolemia syndromes of undetermined etiologies.~~ Modified to: Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia *syndromes as described in Section 2.2.1 and Section 2.1.2, respectively.*	Deletion of redundant text.	Section 2.1 Dyslipidemia
Section 2.1.1 Triglycerides - added text regarding association of severe hypertriglyceridemia and pancreatitis. Modified: "*The risk of acute pancreatitis is elevated with increasing levels of serum triglycerides. Severe hypertriglyceridemia ≥ 500 mg/dl is an established risk factor for acute pancreatitis (Gouni-Berthold et al., 2023). The risk of acute pancreatitis with levels >1000 and >2000 mg/dL is ~5% and 10% to 20%, respectively and control of triglyceride levels to <500 mg/dL can effectively prevent recurrences. Lipid-lowering therapies are a significant component of reduction in risk of recurrence of acute pancreatitis in patients with elevated TG. Although TG levels close to normal may be preferable, levels <500 mg/dL represents a safe therapeutic target for prevention of recurrences (Scherer et al., 2014a).*"	Description of the association of severe hypertriglyceridemia and increased risk of pancreatitis is relevant to Phase 1b disease-specific cohort and added objective.	Section 2.1.1 Triglycerides
Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion – added text regarding rationale for lipid cutoff for severe hypertriglyceridemia in Phase 1b disease-specific cohort expansion. Modified: "*Severe HTG, defined as TG levels of >500 mg/dL (5.65 mmol/L), is a risk factor for ASCVD and is recommended for inclusion in Phase 1b (Gouni-Berthold et al., 2023; Gurevitz et al., 2024). Analyses of characteristics and prevalence of chronic conditions by TG levels have shown that these conditions and multiorgan disease were common at higher TG level, including substantially increased risk of pancreatitis associated with this condition (Gouni-Berthold et al., 2023; Gurevitz et al., 2024; Scherer et al., 2014b).*"	Description of the association of severe hypertriglyceridemia and increased risk of pancreatitis is relevant to Phase 1b disease-specific cohort and added objective.	Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion

Change	Rationale	Affected Section(s)
Section 2.5.5 Clinical Data – New section added with summary of clinical data from subjects treated with CTX310. Modified to: *"Preliminary data from DL 1 (0.1 mg/kg) and DL2 (0.3 mg/kg) indicate a favorable safety profile. CTX310 was well-tolerated with no serious adverse events (SAEs) or serious adverse reactions (SARs), only 1 non-serious related adverse events of special interest (AESI) of rash, no events of infusion-related reactions, clinically relevant abnormal coagulation findings, or events of increased in alanine aminotransferase (ALT) or aspartate aminotransferase (AST): ≥3 × upper limit of normal (ULN) or ≥2 × the baseline value (if baseline ALT ≥ULN), or new malignancy, and no new signals from the safety data review or literature review. All treatment-emergent adverse events (AEs) were mild to moderate in severity. There was no treatment-emergent Grade 3 or higher AE and dose dependent liver enzyme elevations were observed. The benefit-risk profile for CTX310 remains acceptable for continued clinical development."*	Summary and benefit-risk conclusion of available clinical data regarding the safety, tolerability, and clinical activity of CTX310.	Section 2.5.5 Clinical Activity
Section 3 Objectives and Endpoints		
Section 3 Objectives and Endpoint and Section 11.2.3 Exploratory Endpoints– added new Exploratory Endpoint relevant to Phase 1b Modified: "*For Phase 1b only* • *Change from baseline in number of acute pancreatitis events at 12 months in subjects with severe HTG"*	Addition of exploratory endpoint regarding incidence of pancreatitis as secondary measure of clinical effect in subjects with severe HTG.	Section 3 Objectives and Endpoints, Section 11.2.3 Exploratory Endpoints
Section 4 Subject Eligibility		
Section 4.1 Inclusion Criteria and Section 1.1 Protocol Synopsis- Criterion 3 added criterion specific to subjects in Phase 1b with severe HTG Criterion 3: Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>150 mg/dL [1.7 mmol/L]) and/or LDL-C (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for subjects with ASCVD) and/or non–HDL-C (>160 mg/dL [4.1 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L]) at screening, despite treatment. *In Phase 1b cohort expansion part of the study, severe HTG is defined as TG levels of >500 mg/dL (5.65 mmol/L)."*	Addition of lower weight limit and criterion specific to subjects in Phase 1b with severe HTG.	Section 1.1 Protocol Synopsis and Section 4.1 Inclusion Criteria
Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis– delineation of criterion by applicable phase of study and addition of new criterion 29 specific to phase 1b severe HoFH. Modified*: "For Phase 1b only* *29. Treatment with evinacumab within 20 weeks prior to Day 1."*	Added delineation of criterion by applicable phase of study and addition of new criterion specific to phase 1b HoFH.	Section 1.1 Protocol Synopsis and Section 4.2 Exclusion Criteria

Change	Rationale	Affected Section(s)
Section 5 Study Design		
Section 5.1 Investigational Plan and Section 1.1 Protocol Synopsis– added Phase 1b disease-specific cohort expansion with flat dose. Modified to: "*The study is divided into 2 parts (Figure 4); Phase 1a dose escalation followed by Phase 1b disease-specific cohort expansion. Phase 1a will delineate the potential OBD (Section 5.4.2). Phase 1b will confirm the recommended Phase 2 dose (RP2D).*" *The Phase 1a dose escalation part of this study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:* • *Multifactorial chylomicronemia syndrome (MCS).* • *Homozygous familial hypercholesterolemia (HoFH).* • *Heterozygous familial hypercholesterolemia (HeFH)* • *Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).* • *Other HTG/hypercholesterolemia syndromes of undetermined etiologies.* *The Phase 1a dose escalation will include eligible subjects, regardless of underlying dyslipidemia subtype. The majority of subjects enrolled in Phase 1a are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia and HTG. Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk.* *The Phase 1b disease-specific cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD that encompass HTG and/or hypercholesterolemia syndromes in the following disease-specific cohorts:* • *Severe HTG (>500 mg/dL triglycerides)* • *HoFH* • *HeFH* • *Mixed hyperlipidemias (including moderate HTG)* ***Figure 4:*** ***Study Design*** *- new figure added* *"Following completion of eLBW-based dose escalation, the SRC will review the totality of safety and clinical activity data to delineate an OBD. The SRC will also consider approval of a range of flat dose (FD) of CTX310 based on the OBD or the highest tolerated dose tested in phase1a.* *This FD (within the recommended range) will be applied to the phase1b disease expansion cohorts. Safety and clinical activity (e.g., ANGPTL3 reduction) of CTX310 will be evaluated in each of the 4 disease specific cohorts. Each disease specific cohort may enroll up to 12 subjects. Subjects will be dosed concurrently in each disease-*	Added description of Phase 1a subjects and Phase 1b disease-specific cohort expansion with flat dose of CTX310	Section 1.1 Protocol Synopsis and Section 5.1 Investigational Plan

<table>
<tr><th>Change</th><th>Rationale</th><th>Affected Section(s)</th></tr>
<tr><td>specific cohort. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase1a.
“At each Phase 1a dose escalation DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next DL and to Phase 1b disease-specific cohort expansion. Based on analysis of the totality of clinical data and the endorsement of the Data Safety Monitoring Board (DSMB), the sponsor will declare the RP2D.”</td><td></td><td></td></tr>
<tr><td>Section 5.2 Number of Study Subjects, Section 1.1 Protocol Synopsis, and Section 11.4 Sample Size – updated to accommodate addition of Phase 1b disease-specific cohort expansion.
Original: Approximately <s>24</s> eligible subjects will be enrolled in the study.
Modified to: Approximately 69 eligible subjects will be enrolled in the study.
Phase 1a Dose Escalation: Approximately 21 subjects
Phase 1b Cohort Expansion: Approximately 48 (up to 12 subjects per disease-specific cohort).</td><td>Updated sample size required to accommodate addition of Phase 1b disease-specific cohort expansion.</td><td>Section 1.1 Protocol Synopsis and Section 5.2 Number of Study Subjects, Section 11.4 Sample Size</td></tr>
<tr><td>Section 5.4 Dose Escalation and Disease-Specific Cohort Expansion and Section 1.1 Protocol Synopsis– updated section title and edited text to accommodate addition of Phase 1b disease-specific cohort expansion.
Original: “<s>The Sponsor will declare the RP2D at or below the MTD, or alternatively an OBD based on the analysis of clinical data. At least 3 additional subjects will be administered CTX310 at this DL (MTD or OBD) before an RP2D is confirmed. A dose expansion cohort may be added to the protocol in a future amendment. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose level based on predefined criteria provided in a future amendment.</s>
Modified to: “Section 5.4 Dose Escalation and Disease-Specific Cohort Expansion”</td><td>Section title and text were edited to accommodate addition of Phase 1b disease-specific cohort expansion</td><td>Section 1.1 Protocol Synopsis and Section 5.4 Dose Escalation and Disease-Specific Cohort Expansion</td></tr>
<tr><td>Section 5.4.1 Dose Escalation Methodology – added 2 dose levels to Phase 1a
Phase 1a Dose Escalation of CTX310
<table>
<tr><th>Dose Level*</th><th>Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW) [1]</th></tr>
<tr><td>5</td><td>1.0 [3]</td></tr>
<tr><td>6</td><td>1.2 [4]</td></tr>
</table>
[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.
[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.</td><td>Removed additional Phase 1a confirmatory cohort for OBD affirmation and added 2 dose levels</td><td>Section 1.1 Protocol Synopsis and Section 5.4.1 Dose Escalation Methodology</td></tr>
<tr><td>Section 5.4.2 Optimal Biologic Dose Definition – updated section title and edited text to remove MTD
Original: “<s>Maximum Tolerated Dose/Optimum</s> Optimal Biologic Dose Definition
<s>The MTD is the highest dose for which DLTs are observed in fewer than 2 of 6 subject. An MTD may not be determined in this study</s>. The OBD is the lowest dose associated with biological efficacy, as determined by intended decrease in ANGPTL3 levels with minimum toxicity.”</td><td>Section title and text were edited to remove definition of MTD as it is no longer applicable in the study.</td><td>Section 5.4.2 Optimal Biologic Dose Definition</td></tr>
</table>

Change	Rationale	Affected Section(s)
Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions During Phase1a and Phase 1b– updated section header and bulleted definition and Section 1.1 Protocol Synopsis Modified to: 'Dose-limiting Toxicity Rationale and Definitions *During Phase1a and Phase 1b"* Bullets were numbered. Bullet 1 was moved to Bullet 6: Any *other* CTCAE grade ≥3 AE, *other than those listed in bullets #1-5 above,* that is assessed by the investigator as related to investigational product."	Updated section header to specify applicability to Phase1a and Phase 1b. Modified DLT definition to specify criterion applicable to clinical (i.e., non-laboratory results)	Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions During Phase1a Dose Escalation, and Section 1.1 Protocol Synopsis and Section 1.1 Protocol Synopsis
Section 5.4.4 Disease-specific Cohort Expansion – new section Modified to: "*Following completion of eLBW-based dose escalation (Table 3), analysis of PK/PD and safety data, and approval by the SRC, a FD of CTX310 may be evaluated in a Phase 1b cohort expansion in 4 disease-specific cohorts.* *The SRC will endorse the RP2D based on the review of the totality of data including clinical activity and safety of the FD for disease-specific groups. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future protocol amendment or separate study protocol."* Subsequent sections renumbered as necessary.	Added new section to describe the dose determination necessary to accommodate addition of Phase1b disease-specific cohort expansion	Section 5.4.4 Disease-specific Cohort Expansion
Section 5.5.1 Dose Escalation in Phase 1a – previous section (CTX310 Dose Rationale) edited to provide discrete description of dose escalation. Modified to: *"The clinical study with CTX310 will utilize the same LNP formulation used in the CTX310 nonclinical NHP studies, allowing direct correlations between NHP dose and the expected safety and efficacy in human subjects. As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema is based on eLBW, which takes into account sex and height rather than total body weight alone, and, therefore, was selected to reduce the risk of overdosing obese and overweight subjects.* *Exposure data available to date indicate that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Based on available CTX310 PK (data on file), a mean maximal concentration (C_{max}) of 52,800 ± 6,790 ng/mL was observed when assessing of [redacted] exposure following a clinical dose of 0.3 mg/kg CTX310, which is 2.5-fold lower than the C_{max} observed following treatment with 0.5 mg/kg CTX310 in NHPs. Additionally, the [redacted] area under the concentration time curve from time zero to the last quantifiable concentration ($AUCT_{last}$) following a clinical dose of 0.3 mg/kg CTX310 was 1,560,000 ± 654,000 (hr*ng/mL), which was approximately the same as the 0.5 mg/kg CTX310 $AUC_{0\text{-}last}$ exposure in NHP.*	Previous Section 5.5 (CTX310 Dose Rationale) edited to provide discrete description of dose escalation in Phase 1a.	Section 5.5.1 Dose Escalation in Phase 1a

Change	Rationale	Affected Section(s)
Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data."		
Section 5.5.2 Disease-specific Cohort Expansion with Flat Dose in Phase 1b – previous section (CTX310 Dose Rationale) edited to provide discrete description of dose rationale in Phase 1b Modified to: "*Due to the primary distribution of the LNP-mediated CTX310 delivery to the liver, evaluation of safety and clinical activity of FD of CTX310 is considered appropriate and consistent with other liver-targeted genetic medicines that are administered at a FD (Bakris et al., 2024; Coelho et al., 2023; Desai et al., 2023; Young et al., Fontana et al., 2024, NCT06128629, Rat et al., 2020; Srivastava et al., 2023); . Earlier siRNAs (givosiran, lumasiran) initially relied on weight-based dosing (Balwani et al., 2020; Garrelfs et al., 2021) with little difference in efficacy noted regardless of weight. Similarly, in vivo gene editors that target hepatocytes using LNP vehicles likewise began development with weight-based dosing (Gillmore et al., 2021) , but transitioned to flat-dosing in later phases (NCT06128629) and with newer targets (Longhurst et al., 2024) . A similar strategy is planned for Phase 1b, where PK, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts."*	Previous Section 5.5 (CTX310 Dose Rationale) edited to Provide discrete description of dose rationale in Phase 1b	Section 5.5.2 Disease-specific Cohort Expansion with Flat Dose in Phase 1b
Section 6 Study Treatment		
Section 6.1 Administration of CTX310 Revision of duration of infusion Modified: Subjects will receive a single IV infusion ~~within a 1 hour period~~ on Day 1, administered under medical supervision during inpatient hospitalization.	To improve patient safety during infusion, the time allowed for infusion has been extended, as outlined in detail in the Pharmacy Manual.	Section 6.1 Administration of CTX310
Section 6.1.1: Pre-infusion Prophylaxis Added another prophylaxis regimen on Day -1, the evening before CTX310 infusion on Day 1. Added text: *On Day -1, i.e., the evening prior to CTX310 administration on Day 1, an infusion prophylaxis regimen will be administered to subjects as follows:* • *Oral steroid (e.g., dexamethasone approximately 10 mg or equivalent)* Modified the following text: *On Day 1,* within 1 to 2 hours prior to the administration of study drug, an infusion prophylaxis regimen will be administered to subjects ~~The regimen consists of the following~~ *as follows*: • *Optional oral acetaminophen/paracetamol (500 mg to 1000 mg)*	Provide additional prophylaxis regimen prior to infusion	Section 6.1.1 Pre-infusion Prophylaxis Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Section 6.5.2 Prohibited Medication – added prohibited medication necessary to accommodate Phase 1b disease-specific cohort in HoFH. Modified to: "*Phase 1b only: Evinacumab within 20 weeks prior to Day 1 for subjects.*"	Added prohibited medication necessary to accommodate Phase 1b disease-specific cohort.	Section 6.5.2 Prohibited Medications
Section 7.1 General Guidance		
Section 7.1.1 Infusion-Related Reactions – text revised to provide additional background information. Modified to*: Infusion-related reactions have been reported with LNP utilizing treatments, occurring in up to 19% of patients receiving patisiran (Moghimi and Simberg 2022) . Infusion-related reactions can be either allergic reactions to foreign particles or non-immune mediated reactions. Most IRRs are mild and typically develop within minutes to several hours of initiation of the drug infusion, although symptoms may be delayed for up to 24 hours. IRRs may affect any organ system in the body. While most reactions are mild in severity, severe or fatal reactions can occur. The most common signs and symptoms of IRR are fever, chills, flushing, itching, alterations in heart rate (including tachycardia) or blood pressure (including hypotension), dyspnea, chest discomfort, back pain or abdominal pain, nausea, vomiting, diarrhea, and various types of skin rashes.* In the event of an *acute* IRR, the infusion of study drug will be slowed or stopped, and the subject closely monitored until resolution of the reaction…. *Delayed IRR (>12 hours) are usually immune-mediated and respond best to corticosteroid treatment (e.g., methylprednisolone).*	Revised to provide additional mechanistic background information.	Section 7.1.1 Infusion-related Reactions
Section 8 Study Procedures		
Section 8.1.1.5 Long-term Follow-up – modified to include pancreatitis and CV events Modified to: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310 and either discontinue prior to 12 months or complete this study will be asked to participate in a separate LTFU study *for up to 15 years post infusion* to assess long term safety, durability, and *the occurrence of any clinical adverse event, including pancreatitis event and cardiovascular events.* "	Clarified specific events for inclusion in long-term follow-up.	Section 8.1.1.5 Long-term Follow-up
Section 8.1.4 Subject Withdrawal or Discontinuation – added text with explanation of long-term follow-up study. Modified to: "*Subjects who withdraw completely from this study will be asked to participate in the separate long-term follow-up study if the long-term follow-up study has been approved at the investigative site. As CTX320 is not a continuously dosed investigational product, withdrawal from the study due to an AE is not applicable*."	Added text with explanation of long-term follow-up study.	Section 8.1.4 Subject Withdrawal or Discontinuation
Section 8.2.2 Demographics and Medical History – added medical history requirement specific to subjects in Phase 1b with severe HTG. Modified to: "*Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment will be collected*".	Added medical history requirement specific to subjects in Phase 1b with severe HTG.	Section 8.2.2 Demographics and Medical History

Change	Rationale	Affected Section(s)
Section 9 Safety, Adverse Events and Study Oversight		
Section 9.5 Adverse Event Causality – removal of text ~~**Possibly Related**: There is some evidence to suggest a causal relationship between the study treatment or procedure and the AE, but alternative causes also exist.~~ ~~If the relationship between the SAE and the investigational product is determined to be "possible," a rationale for the assessment must be provided by the reporting investigator.~~	Deletion of "Possibly Related" to simplify categorization and improve clarity.	Section 9.5 Adverse Event Causality
Section 9.9 Medication Errors and Overdose – added new section Modified to: "*An overdose or medication error along with any associated adverse event, regardless of seriousness, should be reported to the sponsor on the provided SAE/AESI form within 24 hours of awareness of the medication error/overdose or associated adverse event*." Subsequent sections renumbered as necessary.	In alignment with regulatory requirements and protocol template	Section 9.9 Medication Errors and Overdose
Section 9.10 Pregnancy – added text Modified to: " All pregnancies will be followed through outcome *and the infant will be followed at birth and at 1 year, provided informed consent is obtained.* *Any congenital anomalies and birth defects in the infant should be reported as an SAE. Additionally, any CTX310-related malignancy or death in the infant during the 12-month follow-up period should also be reported as an SAE."*	Revised in alignment with regulatory requirements	Section 9.10 Pregnancy
Section 11 Statistical Analyses		
Section 11.3 Analysis Sets – modified description of DLT evaluable set Modified to: The DLT evaluable set includes all subjects *during dose escalation part* who receive CTX310 infusion and complete the DLT evaluation period or discontinue early after experiencing a DLT."	Modified to provide distinction between Phase 1a and Phase 1b	Section 11.3 Analysis Sets
Section 11.4 Sample Size – updated text to accommodate addition of Phase1b disease-specific cohort expansion. Modified to: "The sample size of the study will be approximately *69* subjects. *This will include approximately 21 subjects in Phase 1a dose escalation and 48 in the phase 1b disease-specific cohort expansion. In Phase 1b, there will be approximately up to 12 subjects in each cohort."*	Updated sample size required to accommodate addition of Phase 1b disease-specific cohort expansion.	Section 11.4 Sample Size and Section 1.1 Protocol Synopsis Number of Subjects
Section 11.6.5 Exploratory Analysis of Pancreatitis Event – new section *"The annualized event rate of pancreatitis after CTX310 will be estimated using Poisson model and compared to baseline to assess the effect of CTX310 on pancreatitis events."* Subsequent sections renumbered.	New section to align with new exploratory endpoint.	Section 11.6.5 Exploratory Analysis of Pancreatitis Event
General		
Footnotes were updated based on additions and deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Minor editorial and formatting changes for consistency were made throughout, as applicable.		

ApoB: apolipoprotein B; AE: adverse event: AESI: adverse event of special interest; ASCVD: atherosclerotic cardiovascular disease; DL: dose level; eLBW: estimated lean body weight; FD: flat dose; GLP: Good Laboratory Practice; HDL-C: high density lipoprotein – cholesterol; HeFH: heterozygous

hypercholesterolemia; HoFH: homozygous hypercholesterolemia; HTG: hypertriglyceridemia; LDL-C: low-density lipoprotein-cholesterol; LNP: lipid nanoparticles; MTD: maximum tolerated dose; NHP: non-human primate; NOAEL: no observed adverse effect level; OBD; optimal biological dose; PD: pharmacodynamic; PK: pharmacokinetic; RP2D: recommended phase 2 dose; SAE: serious adverse event; siRNA: small-interfering ribonucleic acid; SRC: Safety Review Committee; TG: triglycerides;

14.4.2. Summary of Changes to Protocol V5.0, Amendment 4

Summary of Changes to the Current Protocol

Change	Rationale	Affected Section(s)
Changes specific to Section 1.1 Protocol Synopsis (identical changes in the synopsis and the body of the protocol, Sections 2 through 14, are captured in chronological order by protocol body section level heading)		
Section 1.3 Schedule of Assessments, Table 1 Schedule of Assessments		
Section 1.3 Schedule of Assessments – revision of headers to include Day Original: "M2, M3, M6, M9, EOS/M12" Modified to: " M2/*D60*, M3/*D90*, M6/*D180*, M9/*D270*, EOS/M12/*D360*."	Assists in alignment with electronic data capture.	Section1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessments – deletion of echocardiogram on Day 30 and EOS	Modify the requirement for additional echocardiograms to be at the discretion of the investigator.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessment – Additional measurements of CBC Original: Screening, Day 2, Day 4, Day 30, Day 180, EOS Modified to: Screening, *Day 1*, Day 2, *Day 3*, Day 4, *Week 1 Day 7*, *Week 2 Day 14,* Day 30, Day 180, EOS	Additional measurements of CBC to capture adverse events for improved subject safety.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessments; modification to footnote #5 Original: "On Day 1 vital signs should be recorded at the following time points:, prior to the infusion of CTX310, every 15 (± 5 minutes) during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital." Modified: ". On Day 1 vital signs should be recorded at the following time points: *prior to pre-infusion prophylaxis,* prior to the infusion of CTX310, every 15 (± 5 minutes) during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital."	Addition of timepoint for improved subject safety.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Section 1.3 Schedule of Assessments; modification to footnote #7 Original: "~~Echocardiogram is required at screening.~~ Transthoracic echocardiogram will be performed at screening ~~the scheduled visits~~. See Section 8.2.6." Modified to: "Transthoracic echocardiogram *will be performed at screening*. See Section 8.2.6."	Modify the requirements for transthoracic echocardiogram to provide clarity.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessments; modification to footnote #8 Original: "Electrocardiogram: See Section 8.2.7. Day 1 ECG ~~will~~ be collected prior to pre-infusion prophylaxis regimen." Modified: "Electrocardiogram: See Section 8.2.7. Day 1 ECG *can be collected within 24 hours* prior to pre-infusion prophylaxis regimen."	Modification of the timepoint for collection of ECG to provide flexibility.	Section 1.3 Table 1 Schedule of Assessments
Section 2 Introduction		
Section 2.1 Dyslipidemia and Section 1.1 Protocol Synopsis; addition of FCS Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on the following monogenic or polygenic refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes: • Multifactorial chylomicronemia syndrome (MCS). • Homozygous familial hypercholesterolemia (HoFH). • Heterozygous familial hypercholesterolemia (HeFH). • *Familial chylomicronemia syndrome (FCS) (with ≥5% LPL activity).* • Other HTG and/or hypercholesterolemia syndromes of undetermined etiologies.	Modification of the list to align with exclusion criterion #1	Section 2.1 Dyslipidemia and Section 1.1 Protocol Synopsis
Section 2.1.1 Hypertriglyceridemia Original: "As most FCS patients are either LPL-deficient or lack insufficient LPL activity, these patients will likely not benefit from *ANGPTL3*-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and ~~are~~, therefore~~, excluded from this study~~. Modified to: "As most FCS patients are either LPL-deficient or lack sufficient LPL activity, these patients will likely not benefit from ANGPTL3-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and, *therefore, only FCS patients with a documented medical history of ≥5% LPL activity will be included in this study."*	Modification of the study population description to align with exclusion criterion #1	Section 2.1.1 Hypertriglyceridemia

Change	Rationale	Affected Section(s)
Section 2.4 CTX310 – clarification of language Original: "ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; EL hydrolyzes HDL." Modified: "ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; *EL hydrolyzes phospholipids, mainly on HDL particles*."	Expansion of definition to improve accuracy	Section 2.4 CTX310
Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion Original: "~~Although TG~~ levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al, 2020; Watts et al, 2021~~), a higher TG level (≥300 mg/dL [3.4 mmol/L]) has been~~ recommended for inclusion in this ~~study to select for a more severely dyslipidemic patient population~~. Modified to: "*Triglyceride* levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al, 2020; Watts et al, 2021) *and are recommended for inclusion in this study."*	Revised to lower the limit of acceptable triglyceride value and is more inclusive of the intended population.	Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion
Section 4 Subject Eligibility		
Section 4.1 Inclusion Criteria Criterion #1, Synopsis Study Population, Study Design and Study Eligibility, Section 5.1 Investigational Plan, Section 8.1.1 General Study Periods; extension of upper limit of age requirement. Original: "Age of ≥18 and ≤~~70~~ years at the time of signing informed consent." Modified to: "Age of ≥18 and ≤*75* years at the time of signing informed consent."	Modify the eligibility criterion to be more inclusive of upper age limit.	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis and Section 5.1 Investigational Plan
Section 4.1 Inclusion Criteria Criterion #3, Synopsis Study Population qualifying triglyceride level decreased. Original: "Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of triglycerides (>~~300~~ mg/dL [~~3.4~~ mmol/L])…." Modified to: "Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of triglycerides (>*150* mg/dL [*1.7* mmol/L])…."	Revised to lower the limit of acceptable triglyceride value and is more inclusive of the intended population.	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 4.1 Inclusion Criterion #4, Synopsis Study Population modification of definition of 'refractory' Original: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least ~~26~~ weeks prior to screening." Modified to: "Subject lipid levels must be refractory to the *maximal intensity or* MTDs of standard of care lines of *lipid lowering therapies or combinations* where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least *12* weeks prior to screening."	Currently, no patients will be on single line of treatment before being labelled refractory, but will be on combinations of lipid lowering medications. Therefore, the definition of 'refractory' has been revised to encompass all routinely available medications in maximal intensity or MTD. The time it takes to respond to individual medications was evaluated and it was concluded that 12 weeks on maximal intensity /doses of any combination of these medications will be sufficient time to gauge the response	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis
Section 4.1 Inclusion Criterion #6, Synopsis Study Population Original: "Subjects on available standard of care lines of treatment, ~~including but not limited to, statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors~~, must be on a ~~maximum tolerated and~~ stable dose ~~>30 days~~ before screening, with no planned dose increase during the study participation." Modified to: "Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation. "	Revised criterion to require subject be on a stable dose be on a stable dose of treatment to align with standard of care for the intended population.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria Criterion #1 Original: "Subjects with FCS as documented in the medical history." Modified to: "Subjects with FCS *with <5% LPL activity* as documented in the medical history. *If LPL activity testing is not documented, the subject with FCS will be excluded.*	As most FCS patients are either LPL-deficient or lack sufficient LPL activity, they will likely not benefit from CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity. Therefore, only FCS patients with a documented medical history of <5% LPL activity will be excluded.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria – new Criterion #11. New: "*Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm²)*." Criterion #11 through #17 were renumbered to Criterion #12 through 18	New criterion added to improve subject safety	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria – deleted Criterion #18. Original: "~~Current use or use within 90 days from Day 1 of any monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab).~~"	Subjects on monoclonal antibody treatments are not excluded from this Phase 1 study to be more inclusive of the intended population.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria Criterion #25; revised timeframe for prior malignancy Original: "Any prior or current malignancy except for the following that have been completed resected or removed: basal cell carcinoma, squamous cell carcinoma and carcinoma in situ of the cervix or breast, or myeloproliferative disorder or a significant immunodeficiency disorder." Modified to: "Any prior malignancy *within the past 5 years* or current malignancy, *(*except for the following that have been completed resected or removed: basal cell carcinoma, squamous cell carcinoma and carcinoma in situ of the cervix or breast*)*, or myeloproliferative disorder. or a significant immunodeficiency disorder."	Revised timeframe to within past 5 years for prior malignancy to be inclusive of subjects who are in remission.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 5 Study Design		
Section 5.4.1 Dose Escalation Methodology and Section 1.1 Protocol Synopsis - addition of statement referencing dose rationale. Column Modified to: "*The dose rationale based on nonclinical studies in NHPs is described below."* **Table 3 Dose Escalation of CTX310** **Dose Level*** | **Planned Dose *Based on Estimated Lean Body Weight* (mg/kg eLBW)**[1]	Addition of statement referencing Dose Rationale provided for clarity. Column header in Table 2 revised for clarity.	Section 5.4.1 Dose Escalation Methodology, Table 3 Dose Escalation of CTX310, and Section 1.1 Protocol Synopsis
Section 5.4.1 Dose Escalation Methodology and Synopsis Proposed Starting Dose and Dose Escalation Modified to: "*Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future amendment.*"	Addition of option for second dose for eligible subjects once the dose escalation of CTX310 is complete and an RP2D demonstrating clinical benefit and acceptable safety profile is selected	Section 5.4.1 Dose Escalation Methodology and Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 5.4.3 and Protocol Synopsis Dose-limiting Toxicity Rationale and Definition Added new bullets: • *Any CTCAE grade ≥3 elevations in ALT and AST that persist for >14 days and is assessed by the investigator as related to investigational product.* • *Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) that is assessed by the investigator as related to investigational product.* • *Any CTCAE grade ≥3 decrease in platelet count that is assessed by the investigator as related to investigational product.* Original: • Any CTCAE grade ≥3 AE that is related to ~~study drug~~. • "Any CTCAE grade 3 laboratory abnormality that persists ≥7 days and is related to the ~~study drug~~. • Any CTCAE grade 4 laboratory abnormality that is related to the ~~study drug~~." Modified to: • Any CTCAE grade ≥3 AE that is *assessed by the investigator as* related to *investigational product.* • Any *other* CTCAE grade 3 laboratory abnormality that persists ≥7 days and is *assessed by the investigator as* related to the *investigational product*. • Any *other* CTCAE grade 4 laboratory abnormality that is *assessed by the investigator as* related to the *investigational product*."	Addition of new bullets and clarification of bullets regarding laboratory abnormalities to enhance subject safety and capture liver hepatotoxicity, synthetic liver dysfunction.	Section 5.4.3 Dose-limiting Toxicity Rationale and Definition; Section 1.1 Protocol Synopsis
Section 6 Study Treatment		
Section 6.5.2 Prohibited Medications; removal of monoclonal antibodies Original: "~~Monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab)~~"	Subjects on monoclonal antibody treatments are not excluded from this Phase 1 study to be more inclusive of the intended population.	Section 6.5.2 Prohibited Medications

Change	Rationale	Affected Section(s)
Section 8 Study Procedures		
Section 8.2.6 (New number) Echocardiogram -added language Original: "~~Echocardiogram is required at screening~~. A transthoracic echocardiogram for assessment of LVEF will be performed at ~~time points specified in the schedule of assessments~~ (Table 1). Additional echocardiograms may be obtained at the investigator's discretion." Modified to: "A transthoracic echocardiogram *is required at screening* for assessment of LVEF *for all subjects* (see Schedule of Assessments Table 1). Additional echocardiograms may be obtained at the investigator's discretion."	Additional guidelines to enhance subject safety and streamline procedures.	Section 8.2.6 Echocardiogram
Section 8.2.8 Laboratory Tests, Table 4 Local Laboratory Testing Original: "Urinalysis – albuminuria (dipstick) or urine ACR" Modified to: *ACR[1]" "Footnote 1. Albuminuria (dipstick) or ACR to be performed at screening as specified in the schedule of assessments (Table 1)"*	Modified for consistency of language within the program.	Section 8.2.8 Laboratory Tests Table 4 Local Laboratory Testing
Section 9 Safety, Adverse Events and Study Oversight		
Section 9.7 Adverse Event Collection; revision of text Original: "The safety-related information of all subjects ~~enrolled~~ in this study will be recorded from the time of signing the ICF until EOS; however, there are different reporting requirements for the different time periods in the study." Modified to: "The safety-related information of all subjects in this study will be recorded from the time of signing the ICF until EOS/*M12*; however, there are different reporting requirements for the different time periods in the study." Original: "If a subject does not receive CTX310 therapy ~~after enrollment~~, the AE reporting period ends. ~~30 days after last study-related treatment or procedure (e.g., pretreatment regimen, imaging)~~." Modified to: "If a subject does not receive CTX310 therapy, the AE reporting period ends."	Editorial revision for improved clarity	Section 9.7 Adverse Event Collection Period

Change	Rationale	Affected Section(s)
Section 10 Stopping Rules and Study Termination		
Section 10.1 Stopping Rules for the Study– modify language Original: "~~The occurrence of laboratory results meeting any of the criteria in Section 10.1.1 will result in immediate suspension of accrual to the study for safety reasons pending review of the data by the sponsor, SRC, and DSMB~~. Modified to: "*Administration of investigational product will be paused temporarily for safety reasons, pending review of the data by the sponsor, SRC, and DSMB, upon the occurrence of laboratory results meeting any of the criteria in Section* 10.1.1."	Revision of language to clarify temporary nature of suspension while under review.	Section 10.1 Stopping Rules for the Study
Section 10.1.1 Stopping Rules – modification of rules 1 to 3 and reordering of numbers to accommodate addition. Original: "Study accrual will be ~~suspended~~ as described in Section 10.1 if any of the following criteria are met: 1. ALT or AST >8 × ULN, which is confirmed by repeat testing. 2. ALT or AST >5 × ULN, which is confirmed and persists for >~~4 weeks~~. 3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 persisting for >~~2 weeks~~. 4. Fatal or life-threatening AE that is due to ~~study drug~~. Modified to: *Treatment of the next subject* will be *paused* as described in Section 10.1 if any of the following criteria are met: 1. ≥*2 subjects at a DL with* ALT or AST >8 × ULN, which is confirmed *and persists for >14 days*. 2. ≥*2 subjects at a DL with* ALT or AST >5 × ULN, which is confirmed and persists for *>30 days.* 3. ≥*2 subjects at a DL with* ALT or AST >3× ULN (or the greater of 2× baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 × *ULN* persisting for *>14 days*. 4. Fatal or life-threatening AE that is due to *investigational product.*	To improve alignment with expected hepatotoxicities, specified that the treatment of the next subject will be paused if the first 3 stopping rule criteria are met in ≥2 subjects at a dose level. Added the duration of persistence of ALT or AST elevations ≥8×ULN for the first rule.	Section 10.1.1 Stopping Rules for the Study

Change	Rationale	Affected Section(s)
Section 11 Statistical Analyses		
Section 11.3 Analysis Sets – added definition of DLT Evaluation Set and modification of definition of Full Analysis Set New: "*The DLT evaluable set includes all subjects who received CTX310 infusion and complete the DLT evaluation period or discontinue after experiencing a DLT. The DLT period will begin with CTX310 infusion and last for 30 days, or beyond for improvement or resolution of the signs and symptoms of a potential DLT. The DLT evaluable set will be used for dose escalation decisions.*" Original: "The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment ~~or discontinue earlier~~. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL." Modified to: "The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL."	Addition of the definition of the DLT evaluable set needed to support a complete data analysis.	Section 11.3 Analysis Sets
Section 14 Appendix: Definition and Supporting Operational Details		
Section 14.2 Contraception Requirements – added text New: "*Note: Male condoms without spermicide may be used with another acceptable method of female contraception listed below (see acceptable contraceptive methods for female partners of male subjects).*"	Added text for clarification	Section 14.2 Contraception Requirement
Section 14.2 Contraception Requirements – revision of text Original: "Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of ~~mobilization~~ and 1 barrier method of contraception." Modified to: "Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of *enrollment* and 1 barrier method of contraception."	Clarification of timepoint	Section 14.2 Contraception Requirement

Change	Rationale	Affected Section(s)
General		
The term "preclinical" was replaced with "nonclinical' throughout the document for consistency. Footnotes were updated based on additions and deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Minor editorial and formatting changes for consistency throughout, as applicable.		

ALT: alanine aminotransferase; AST; aspartate aminotransferase; CBC: complete blood count; CRF: case report form; CTCAE: Common Terminology Criteria for Adverse Events; DSMB: Data Safety Monitoring Board; DL: dose level; eLBW: estimated lean body weight; EOS: end of study; ICF: informed consent form; INR: international normalized ratio; M12: month 12; MRE: magnetic resonance elastography; MRI: magnetic resonance imaging; NHP: non-human primates; mPDFF: protein density fat fraction; SRC: Safety Review Committee; ULN: upper limit of normal.

14.4.3. Summary of Changes to Protocol V4.0, Amendment 3

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments: Removed the table row and Footnote #7 for Cardiac Stress Imaging ~~*"Cardiac stress imaging with echocardiography or rMPI (SPECT or PET) will be performed at screening. See Section 8.2.6 for details."*~~ The Footnote numbering has been adjusted.	The cardiac stress imaging was added in the prior amendment by the sponsor as an additional assessment to enable assessment of clinically relevant ASCVD during enrollment. However, this assessment may impact inclusion of the intended study population in this clinical study. Other cardiac assessments, e.g., echocardiogram and ECG assessments have already been included for decision regarding eligibility	Section 1.3, Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #1 and Section 8.1.1.1 Screening and Enrollment: removed mention of cardiac stress imaging Modification shown in strikethrough: "All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, ~~cardiac stress imaging,~~ and infectious disease markers (if these procedures were performed within 60 days prior to infusion)."		Section 1.3, Table 1 Schedule of Assessments, Section 8.1.1.1.
Protocol Synopsis and Section 4.2 Exclusion Criteria: Removed Exclusion Criterion #28. *"~~28.Abnormal finding of myocardial ischemia on cardiac stress imaging (stress echocardiogram or radionuclide myocardial perfusion imaging [rMPI]) at screening. If results are inconclusive, findings should be discussed with the Safety Review Committee (SRC) prior to enrollment~~."* The downstream Exclusion Criterion numbering has been adjusted and the total number of exclusion criteria has changed from 29 to 28.		Section 1.1 Protocol Synopsis; Section 4.2 Exclusion Criteria
Section 8.2.6 Cardiac Stress Imaging: removed and downstream level 3 subheadings have been adjusted accordingly. *"~~8.2.6 Cardiac Stress Imaging~~* ~~*A stress echocardiogram or rMPI (using single-photon emission computed tomography [SPECT] or positron emission tomography [PET]) will be performed before CTX310 administration at screening as described in the schedule of assessments (Table 1). Exercise or a pharmacologic agent may be used to induce stress conditions. Standard local regulations will be used to image acquisition and interpretation. During screening, if the results of stress echocardiogram or rMPI are not clearly interpretable for a subject, the sponsor may approach the SRC for a decision regarding eligibility.*~~"		Section 8.2.6 Cardiac Stress Imaging. Downstream level 3 section heading numberings updated

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments current Footnote #7 for Echocardiogram and Section 8.2.7 (renumbered to 8.2.6) Echocardiogram: removed mention of cardiac stress imaging. Modification shown in strikethrough: Echocardiogram is required at screening. A transthoracic echocardiogram (for assessment of LVEF) will be performed at the time points specified in the schedule of assessments (Table 1). ~~unless done as part of cardiac stress imaging. The LVEF obtained during the resting portion of a stress echocardiogram (Section 8.2.6) may be used for screening purposes~~. Additional echocardiograms may be obtained at the investigator's discretion.		Section 1.3, Table 1 Schedule of Assessments, Section 8.2.6 Echocardiogram (section renumbered)
Schedule of Assessments: added a new row: for cardiac biomarker test prior to infusion of CTX310 and added reference to Footnotes #11 and #12 Section 6.1 Administration of CTX310 *Prior to administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor (see Table 1).* Also replicated this statement in Schedule of Assessment Footnote #12 Section 8.2.8 Laboratory Tests and Table 4 Local Laboratory Tests *Cardiac biomarker* | *Troponin I or T*	Tests for elevation of cardiac biomarker as a safety measure prior to CTX310 administration	Section 1.3 Table 1 Schedule of Assessments, Section 6.1 Administration of CTX310, Section 8.2.8 Laboratory Tests Table 4 Local Laboratory Tests
Modified Inclusion Criterion #4 to remove the ANGPTL3 inhibitor, evinacumab, from the list. Deletion is shown in Strikethrough in modified Inclusion Criterion #4: Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab) ~~or ANGPTL3 (evinacumab~~), for at least 26 weeks prior to screening.	Evinacumab (Evkeeza®) is not available or has only recently been approved in some countries (e.g., Health Canada approval on 25 September 2023) and subjects may not be able to meet the criteria of refractoriness to maximum tolerated dose (MTD) for at least 26 weeks prior to screening.	Section1.1 Synopsis – Inclusion Criteria; Section 4.1

Change	Rationale	Affected Section(s)
Modified Exclusion Criterion 3 as shown in italics: Complete blood count: neutrophils <1000 cells/µL (1.0 × 10^9/L); lymphocytes <500 cells/µL (0.5 × 10^9/L); hemoglobin <11 g/dL (~~6.83mmol~~ *110 g/L*) for males, <10 g/dL (~~6.21 mmol~~ *100 g/L*) for females; or platelet count <100,000/µL (100 × 10^9/L).	Replaced unit of measurement for hemoglobin in mmol with standard international (SI) unit.	Section 1.1 Protocol Synopsis; Section 4.2
Updated current Footnote #18 in the Schedule of Assessments (Table 1) and applied clarifications in Section 8.4.1 for consistency. Original: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, D2, D3, and D4 (48-, 72-, and 96-hours post-infusion time points, respectively), a single sample will be collected (Section 8.4.1)." Modified to: "PK testing: *See Section 8.4.1*: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 *(±5 min)*, 2 *(±5 min)*, and 7 *(±15 min)* hours after completion of CTX310 infusion. On D2, D3, and D4 (*24 [±2 hours]-, 48 [±2 hours] -, and 72 [±2 hours]* -hours post-infusion time points, respectively), a single sample will be collected. A single sample will also be collected for all other scheduled timepoints." Updated Section 8.4.1 to align with the Schedule of Assessments and consolidate the windows for timepoints in Table 1 Footnote #18. Original: "On Day 1, samples will be collected at prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after the completion of CTX310 infusion. For all other time points, D2, D3, and D4 (48, 72, and 96 hours post-infusion time points, respectively), a single sample will be collected." Modified to: On Day 1, samples will be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at the scheduled time points after the completion of CTX310 infusion, *as described in the schedule of assessments (Table 1)*. For all other time points specified *in the schedule of assessments (Table 1)*, including D2, D3, D4, a single sample will be collected as described.	Added windows for timing of PK sample collection and corrected the hours of the timepoints corresponding to D2, D3, and D4.	Section 1.3 Table 1 Schedule of Assessments, Section 8.4.1

Change	Rationale	Affected Section(s)
Updated current Footnote # 22 in the Schedule of Assessments (Table 1). Original: "Serum samples for exploratory biomarker assessments (e.g., cytokines): On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, a single sample will be collected (Section 8.4.3.3)." Updated Footnote #22 Modified to: "Serum samples for exploratory biomarker assessments (e.g., cytokines*): See Section 8.4.3.3:* On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 *(±5 min),* 2 *(±5 min)*, and 7 *(±15 min)* hours after completion of CTX310 infusion. *On D2 and D3 (24 [±2 hours]-, and 48 [±2 hours] -hours post-infusion time points, respectively)*, a single sample will be collected."	Added windows for timing of sample collection and corrected the hours of the timepoints corresponding to D2 and D3.	Section 1.3 Table 1 Schedule of Assessments

Glossary of Terms in the appendix was updated. Section numbering, footnotes were updated based on deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Editorial changes were applied for clarification, as needed.

14.4.4. Summary of Changes to Protocol V3.0, Amendment 2

Change	Rationale	Affected Section(s)
Schedule of Assessments		
Table 1 Schedule of Assessments Footnote #1; added text "*Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, cardiac stress imaging, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).*"	Addition of text to the schedule of assessment Footnote 1 for clarity	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #3; clarified text Original: "All participants who receive an infusion of CTX310, including those who terminate the study prior to 12 months, will be asked at the EOS visit to sign an informed consent for rollover into a separate long-term follow-up study for up to 15 years post-infusion (Section 8.1.1.5)." Modified to: "All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months and those who complete the study, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion (Section 8.1.1.5)."	Clarified that the subjects who discontinue the study prior to M12 will be asked to consent to roll over into a separate long-term follow-up study for up to 15 years after post-infusion.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #4; added D1 Original: "Complete physical exam required at screening, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3). Modified to: "Complete physical exam required at screening, *D1*, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3)."	Added a full physical exam on Day 1	Section 1.3 Schedule of Assessments, Table 1 Section 8.2.3 Physical Examination, Height and Weight
Table 1 Schedule of Assessments Footnote #5; added text. Original: "On Day 1 vital signs should be recorded at the following time points: prior to the infusion of CTX310, every 15 minutes during the infusion, at 1, 2, and 5 hours after the end of the infusion, and then every 8 hours until discharge from the hospital." Modified: "On Day 1 vital signs should be recorded at the following time points: prior to the infusion of CTX310, every 15 ± *5* minutes during the infusion, at 1, 2, *3, 6 hours (± 15 minutes), and 8* hours (± *30 minutes)* after the end of the infusion, and then every 8 hours (± *30 minutes)* until discharge from the hospital."	Increased the number of monitoring time points and added windows for monitoring for vital signs post-infusion on Day 1 and Day 2.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments: Added a new Footnote #7 for Cardiac Stress Imaging "Cardiac *stress imaging with echocardiography or rMPI (SPECT or PET) will be performed at screening.* See Section 8.2.6 for details." The downstream Footnote numberings have been adjusted (e.g., Original Footnote #7 has been updated to Footnote #8: : "Echocardiogram: See Section 8.2.7 for details) A new Section, Section 8.2.6, has been added for cardiac stress imaging and downstream level 3 headings have been adjusted accordingly	The cardiac stress imaging will enable assessment of clinically relevant ASCVD during enrollment.	Section 1.3 Table 1 Schedule of Assessments, Section 8.2.6 Cardiac Stress Imaging
Table 1 Schedule of Assessments Footnote #8 Original: [8]Echocardiogram: "Transthoracic echocardiogram will be performed at the scheduled visits. See Section 8.2.7 for details." Modified: [8]Echocardiogram: "Transthoracic echocardiogram will be performed at the scheduled visits. See Section 8.2.7 for details. *Required at screening unless done as part of cardiac stress imaging. The LVEF obtained during the resting portion of a stress echocardiogram (Section 8.2.6) may be used for screening purposes."*	Added text to allow for LVEF obtained during the stress echocardiogram to be used for screening purposes.	Section 1.3 Table 1 Schedule of Assessments Section 8.2.7 Echocardiogram
Table 1 Schedule of Assessments Footnote #9. Original: "ECG: See Section 8.2.7." "ECG: See Section 8.2.8. *Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen.*"	Clarification that Day 1 ECG will be collected prior to the pre-infusion prophylaxis regimen.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #14; removed D1 pregnancy test requirement. Table footnote 10 redundant text deleted. Original: "Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment. ~~Females of childbearing potential must be excluded from the study. Females with a uterus must be postmenopausal, defined by at least 12 consecutive months of amenorrhea without an alternative medical cause prior to screening, or surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, or oophorectomy at least 1 month prior to screening~~). " Modified to: "Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment.~~"~~	Pregnancy test at Day 1 is not required as it will be performed at screening, as per local standard, for this trial that will enroll women who meet the criteria of being non-childbearing. Redundant text deleted from Table 1 Footnote 10.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments footnote 14 revised. Original: "All females ~~enrolled in the study~~ must have a negative pregnancy test ~~within 72 hours prior to CTX310 infusion~~ (Section 8.2.8, Table 4, and Section 8.2.9)." Modified to: "*At screening*, all females must have a negative *serum* pregnancy test *performed as per local standard* (Section 8.2.9Table 4, and Section 8.2.10)."		
Table 1 Schedule of Assessments Footnote #13; clarified D1 assessment Original: "See listings of laboratory assessments (Table 4 and Table 5) for details. On Day 1 laboratory values should be performed prior to the infusion of CTX310. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to define AESI (Section 9.3) and stopping rules (Section 10.2)." Modified to: "See listings of laboratory assessments (Table 4 and Table 5) for details. Day 1 laboratory *assessments* should be performed *within 24 hours* prior to the infusion of CTX310. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to *monitor* AESI (Section 9.3) and stopping rules (Section 10.2)."	Added to provide clinical sites a flexible time window to perform local laboratory assessments prior to CTX310 infusion.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #15; clarified genetic testing for rescreen. Original: "The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.8 and Table 5 for details." Modified to (italicized text also added as new footnote 1 to Section 8.2.9 Table 5): "The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.9 and Table 5 for details. *Collection of a sample for genetic testing should not be repeated during rescreening, if applicable*."	Clarification of the need for a single sample for genetic testing.	Section 1.3 Table 1 Schedule of Assessments, Section 8.2.9 Laboratory Tests Table 5 Central Testing
Table 1 Schedule of Assessments Footnote #19; clarified timing of PK assessments. Original: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points a single sample will be collected (Section 8.4.1)." Modified to: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, *D2, D3, and D4 (48-, 72-, and 96-hours post-infusion time points, respectively),* a single sample will be collected (Section 8.4.1)."	Clarification of time points at with requirement for a single sample.	Section 1.3 Table 1 Schedule of Assessments Section 8.4.1 CTX310 Pharmaco-kinetic Analysis

Change	Rationale	Affected Section(s)
Introduction		
Section 2.2 ANGPTL3 Original: "~~ANGPTL3 regulates plasma TG levels by inhibiting LPL, which is the key enzyme responsible for the breakdown or hydrolysis of TG into free fatty acids (FFA) and glycerol. ANGPTL3 also inhibits endothelial lipase (EL), which is primarily responsible for the lipolysis of HDL-C. In addition to its role in regulating TG levels, mice and humans with mutant ANGPTL3 or inactivation of ANGPTL3 rapidly clear VLDL in an independent manner (Adam et al, 2020; Musunuru et al, 2010; Shimizugawa et al, 2002; Wang et al, 2015). VLDL is a precursor byproduct for the production or metabolism of LDL-C, hence its clearance limits the production of LDL-C. Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Dewey et al, 2017) . ANGPTL3 inhibition also substantially lowers ApoB levels, which has been shown to proportionally decrease CVD risk(Ference et al, 2019)~~ ." Modified to: "*ANGPTL3* regulates plasma lipid levels through inhibition of LPL and endothelial lipase (EL). Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Pouwer et al, 2020). *ANGPTL3* LOF variants have been associated with decreased levels of TG, LDL-C, non-HDL-C, ApoB, and HDL-C, as well as a 41% lower risk of coronary artery disease (Dewey, et al 2017). Mechanistic studies indicate that ANGPTL3 inhibition leads to clearance of VLDL remnant particles, upstream of LDL formation, and that LDL-C lowering with ANGPTL3 inhibitors is independent of LDL receptor function (Adam et al, 2020; Reeskamp et al, 2021; Wu et al, 2020)."	Clarification of text	Section 2.2 ANGPTL3
Study Design		
Update to Investigational Plan to align with the dose escalation methodology. Section 5.1 Investigational Plan, Paragraph 7. Original: "~~Once dose escalation is completed and a RP2D has been determined, at least 3 additional participants will be enrolled at the same dose level to confirm safety and pharmacodynamic (PD) effect (confirmatory cohort)~~." Modified to: "*The sponsor will declare the RP2D at or below the MTD, or alternatively an optimum biological dose (OBD) based on the analysis of clinical data. At least 3 additional subjects will be administered CTX310 at this DL (MTD or OBD) before an RP2D is confirmed. A dose expansion cohort may be added to the protocol in a future amendment.*"	Alignment of language with Section 5.4.1 Dose Escalation Methodology for clarity.	Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
Section 1.1 Protocol Synopsis Study Population and Section 5.1 Investigational Plan: Original: "The study population will consist of subjects 18 to 70 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride (TG) and/or non–high-density lipoprotein cholesterol (HDL-C), including low-density lipoprotein (LDL), ~~very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) (Lp(a)),~~ and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTD) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population)." Modified to: "The study population will consist of subjects 18 to 70 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride(s) (TG) and/or non–high-density lipoprotein cholesterol (non-HDL-C), including low-density lipoprotein cholesterol (LDL), and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTDs) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population)."	Clarification of the description of the study population.	Section 1.1, Protocol Synopsis, Section 5.1 Investigational Plan
Section 1.1 Protocol Synopsis Dose Escalation – addition of footnoted statement regarding addition of intermediate dose level(s). "* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose."	Addition of statement supporting addition of intermediate dose level(s) to support the safe execution of the trial	Section 1.1, Protocol Synopsis, Dose Escalation of CTX310 table and Dose Limiting Toxicity Assessment, Section 5.4.1 Dose Escalation Methodology Table 3 Dose Escalation of CTX310

Change	Rationale	Affected Section(s)
Study Objectives and Endpoints		
Synopsis Objectives and Endpoints, Section 3 Study Objectives and Endpoints, Section 11.1, and Section 11.2.1 Primary Endpoints. Original: "Incidence of AEs, including TEAEs, AESIs, DLTs; clinically significant laboratory abnormalities; and clinically significant abnormal vital signs" Modified to: "Incidence of ~~AEs, including TEAEs, AESIs,~~ DLTs; ~~clinically significant laboratory abnormalities; and clinically significant abnormal vital signs~~" New Secondary Objective in Synopsis and Section 3 Study Objectives and Endpoints: "*To further characterize the safety of CTX310*" New Secondary Endpoint in Synopsis, Section 3 Study Objectives and Endpoints, and Section 11.2.2.2 Secondary Safety Endpoint: "*Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs*"	Alignment of primary endpoint with the primary study objective and addition of a secondary objective and corresponding endpoint to further characterize the safety of CTX310.	Section 1.1, Synopsis Objectives and Endpoints, Section 3 Study Objectives and Endpoints, Section 11.1 Study Objectives and Hypotheses, Section 11.2.1 Primary Endpoints, Section 11.2.2.2 Secondary Safety Endpoints
Subject Eligibility		
Inclusion Criterion #4 addition of fibrates and omega-3 Original: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, icosapent ethyl, and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening." Modified to: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, *fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants,* ~~icosapent ethyl,~~ and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening."	Addition of fibrates and omega-3 as each is considered a standard of care	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria
Inclusion Criterion #5 removal of "homozygous familial hypercholesterolemia" Original: "Participants ~~with homozygous familial hypercholesterolemia~~ receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening." Modified to: "Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening."	Clarification that Inclusion Criterion #5 is not intended for subjects with homozygous familial hypercholesterolemia. This was a typographical error and does not impact any other section of the protocol.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria

Change	Rationale	Affected Section(s)
Inclusion Criterion #6 addition of "but not limited to" Original: "Subjects on available standard of care lines of treatment, including statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors, must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation." Modified to: "Subjects on available standard of care lines of treatment, including, *but not limited to* statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors, must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation."	Addition of text to allow for more options of standard of care.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria.
Inclusion Criterion #7 addition of "except as required by the protocol." Original: "Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation. " Modified to: "Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation *except as required by the protocol."*	Clarification that changes to the apheresis schedule are allowed during the study as described in Section 6.5.1.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria.
Inclusion Criterion #11, removal of requirement for participation in a long-term follow-up study as an inclusion criterion. Deleted Inclusion Criterion #11 ~~Willing to participate in a long-term follow-up study for up to 15 years after completion of this study.~~	The requirement for up to a 15-year follow-up after CTX310 infusion has already been built into the study design and in Section 8.1.1.5 and need not be an eligibility criterion. The subjects will be asked to participate in a separate LTFU study for up to 15 years post-infusion.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria
Exclusion Criterion #3 addition of neutrophil and lymphocyte cell count Original: Complete blood count: ~~White blood cell <2,500 cells/µL (2.5 × 10^9/L);~~ hemoglobin <11 g/dL (6.83 mmol/L) for males, <10 g/dL (6.21 mmol/L) for females; or platelet count <100,000/µL (100 ×10^9/L). Modified to: "Complete blood count: *neutrophils <1000 cells/µL (1.0 × 10^9/L); lymphocytes <500 cells/µL (0.5 × 10^9/L)*; hemoglobin <11 g/dL (6.83 mmol/L) for males, <10 g/dL (6.21 mmol/L) for females; or platelet count <100,000/µL (100 ×10^9/L)."	Instead of white blood cell count, specified neutrophil and lymphocyte count for exclusion of subjects with neutropenia and lymphocytopenia per se.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria

Change	Rationale	Affected Section(s)
Exclusion Criterion #5 addition of testing result requirements. Original: "Diagnosis of nephrotic syndrome" Modified to: "Diagnosis of nephrotic syndrome *or albuminuria >2+ on urine dipstick or albumin to creatinine ratio of >300 mg/g.*"	Addition of testing result requirements added for clarification.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Exclusion Criterion #7 modification of text Original: "History of alcohol or drug abuse." Modified to: "History of alcohol or *substance use disorder*."	Revision of language to accommodate diagnosis description as per DSM-5	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Exclusion Criterion #20 clarification; removal of selective serotonin reuptake inhibitor Original: "Current use of ~~selective serotonin reuptake inhibitors,~~ chronic systemic corticosteroid therapy, or anabolic agents." Modified to: "Current use of chronic systemic corticosteroid therapy, or anabolic agents."	Removal of selective serotonin reuptake inhibitors from the list of prohibited medications as their use will have minimal effect on assessment of lipid levels post-CTX310 administration as the subjects will continue SSRIs during the course of the study	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria,
Exclusion Criterion #21 clarification Original: "Current use of niacin-based supplements or nutraceuticals that may influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit." Modified to: "Current use of *nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly* influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit."	Addition of text to include both niacin-based or other nutraceuticals known to significantly influence lipid levels	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria Section 6.5.2 Prohibited Medications
Exclusion Criterion #23 clarification Original: "Positive serology for HIV-1 or HIV-2, hepatitis B virus (hepatitis B core antibody or NAT), or hepatitis C virus (NAT). Modified to: "Positive serology for HIV type 1 *or type 2,* hepatitis B virus (hepatitis B core antibody *or hepatitis B surface antigen or NAT*), or hepatitis C virus (*hepatitis C antibody testing* or NAT*)*."	Addition of options for testing to allow flexibility to clinical sites	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria

Change	Rationale	Affected Section(s)
Exclusion Criterion #25 clarification Original: "Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder." Modified to: "Any prior or current malignancy *except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ, and carcinoma in situ of cervix or breast,* or myeloproliferative disorder or a significant immunodeficiency disorder."	Addition of text to account for the high prevalence of skin cancer in Australia and New Zealand and the curability following full resection	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Addition of new Exclusion Criterion #28 "Abnormal finding of myocardial ischemia on cardiac stress imaging (stress echocardiogram or radionuclide myocardial perfusion imaging [rMPI]) at screening. If results are inconclusive, findings should be discussed with the Safety Review Committee (SRC) prior to enrollment."	Addition of a new exclusion criterion for additional safety screening for ASCVD prior to infusion of CTX310	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Addition of new Exclusion Criterion #29 *Administration of vaccines 30 days before CTX310 infusion.*	Addition of a new exclusion criterion in alignment with the prohibition of vaccine administration 30 days before CTX310 infusion.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Study Procedures		
Section 1.2 Study Schema, new footnote 'c': "[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for rollover into a separate LTFU study for up to 15 years post-infusion."	Clarified the informed consent signature for the long-term follow-up study.	Section 1.2 Study Schema
Section 6.1 Administration of CTX310: addition of text regarding criteria for eligibility for administration of CTX310 on Day 1. Added text: "*Prior to administration of CTX310 on Day 1, investigator should confirm the ability of the subject to receive the infusion (see Table 1) by ensuring:* • *No significant change in clinical status since screening.* • *No new, clinically significant findings seen on physical exam, vital signs or ECG.* • *No AST, ALT, total bilirubin >2 × ULN and PT (INR) >1.5 × ULN.* *If the infusion is delayed for more than 30 days, the subject will be replaced if deemed necessary for dose escalation decisions*."	Addition of text to confirm eligibility	Section 6.1 Administration of CTX310

Change	Rationale	Affected Section(s)
Section 6.5.1 Allowed Medications and Procedures; added text regarding use of SSRIs and HRT *"Subjects previously prescribed selective serotonin reuptake inhibitors (SSRIs) or hormone replacement therapy (HRT) should remain on a stable dose from at least 30 days prior to screening through the end of the study."*	Addition of text to allow stable SSRI or HRT dosing.	Section 6.5.1 Allowed Medications and Procedures
Section 6.5.1 Allowed Medications and Procedures Original: "The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3, ~~icosapent ethyl,~~ evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study." Modified to: "The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3 *(e.g., ethyl esters of EPA or DHA), bile acid sequestrants*, evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study.	Added examples of omega-3 supplements for clarity	Section 6.5.1 Allowed Medications and Procedures
Section 6.5.2 Prohibited Medications: deletion of SSRIs • ~~Selective serotonin reuptake inhibitors.~~ Addition of prohibition of vaccine administration and oral anticoagulants in the time period proximate to administration of study drug. *"All oral anticoagulants (e.g., warfarin, apixaban, rivaroxaban, or dabigatran) should not be administered 30 days before or 30 days after infusion of CTX310."* *"Vaccines should not be administered 30 days before or 30 days after infusion of CTX310."*	SSRIs are allowed at a constant dose – see Section 6.5.1. Vaccines and oral anticoagulants should not be administered 30 days before or after infusion of CTX310.	Section 6.5.2 Prohibited Medications
Section 6.5.2 Prohibited Medications: Rearranged the statement and added fish oil or red yeast rice as examples of nutraceuticals. ~~Niacin or~~ Nutraceuticals *(e.g., niacin*-based supplements or ~~nutraceuticals~~ *fish oil or red yeast rice)* that ~~may~~ *significantly* influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.	Addition of examples of nutraceuticals that may alter lipid levels.	Section 6.5.2 Prohibited Medications
Section 6.6 Lifestyle Considerations Addition of prohibition of any tissue/blood donation for the duration of the study. *Subjects who receive CTX310 must not donate eggs, sperm, blood, or organs for the duration of this study.*	Addition of prohibition of any tissue/blood donation for the duration of the study.	Section 6.6 Lifestyle Considerations

Change	Rationale	Affected Section(s)
Section 8.1.1.1 Screening and Enrollment; added "Cardiac Stress Imaging" Original: "Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, echocardiogram, and infectious disease markers (if these procedures were performed within 60 days prior to infusion)." Modified to: "Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-*PDFF*/ultrasound of liver, *cardiac stress imaging,* echocardiogram, and infectious disease markers (if these procedures were performed within 60 days prior to infusion)."	Added stress imaging to the list of screening that do not need to be repeated upon rescreening within 60 days prior to infusion	Section 8.1.1.1
Section 8.1.1.5 Long-term Follow-up; clarified text Original: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310, and either discontinue or complete this study, will be asked to participate in a separate LTFU study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events." Modified to: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310, and either discontinue *prior to 12 months* or complete this study, will be asked to participate in a separate LTFU study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events. "	Clarified the text describing LTFU to include those who discontinue prior to12 months	Section 8.1.1.5 Long-term Follow-up
Section 8.2.1 Informed consent, new text added: "*All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to rollover into a separate LTFU study for up to 15 years post-infusion (see Section 8.1.1.5 and Table 1*)."	Clarified the informed consent signature for the LTFU study.	Section 8.2.1 Informed Consent
Section 8.2.3 Physical Examination, Height, and Weight Original: "Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, Day 30, and EOS visits, and the results documented." Has been modified to: "Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, *Day 1*, Day 30, and EOS visits, and the results documented."	Added a full physical exam on Day 1	Section 8.2.3 Physical Examination, Height and Weight

Change	Rationale	Affected Section(s)
New Section 8.2.6 Cardiac Stress Imaging; added new text *A stress echocardiogram or rMPI (single-photon emission computed tomography [SPECT] or positron emission tomography [PET]) will be performed before CTX310 administration at screening as described in the schedule of assessments (Table 1). Exercise or a pharmacologic agent may be used to induce stress conditions. Standard local regulations will be used to image acquisition and interpretation. If the results of the stress imaging are not clearly interpretable for the subjects, the sponsor may approach the SRC for a decision regarding eligibility.*	Additional safety screening for ASCVD prior to infusion of CTX310	Section 8.2.6 Cardiac Stress Imaging
Section 8.2.8 Electrocardiogram; Addition of requirement for Day 1 ECG prior to CTX310 infusion. Original: "Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion." Modified to: "Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). *Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen.* QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion."	Clarification that Day 1 ECG will be collected prior to the pre-infusion prophylaxis regimen.	Section 8.2.8 Electro-cardiogram
Section 8.2.9 Laboratory Tests; clarification of assessments Serum chemistry: Original: "ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein" Modified to: ""ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN/*urea*, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein" Urinalysis: Original: "Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal)" Modified to: "Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal), *albuminuria (dipstick) or urine ACR"*	Clarification of laboratory testing to be performed at the local level.	Section 8.2.9 Laboratory Tests, Table 4 Local Laboratory Testing, Table 5 Central Testing

Change	Rationale	Affected Section(s)
Coagulation Original: "PT, PTT, fibrinogen" Modified to: "PT *(INR),* PTT/*aPTT*, fibrinogen" CBC with differential: Original: "Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count" Modified to: "Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count, *absolute lymphocyte count. Note: The white blood cell count differential may be reported as absolute counts or as percentages, with the exception that absolute neutrophil and lymphocyte counts are required for eligibility assessment* Viral serology Original: "HIV-1, HIV-2, HCV antibody ~~and RNA~~, HBV surface antigen, HBV surface antibody, HBV core antibody" Modified to: "HIV-1, HIV-2, HCV antibody *or NAT*, HBV surface antigen, HBV surface antibody, HBV core antibody *or NAT"* Table 5 Central Testing Footnote 1 added: "*Collection of a sample for genetic testing should not be repeated during rescreening, if applicable."*		
Section 8.2.10 deletion of "~~enrolled in the study~~" for consistency with Table 1 footnote and "~~A negative serum pregnancy test, performed as per local standards, is required within 72 hours before CTX310 infusion~~."	Pregnancy test at Day 1 is not required as it will be performed at screening, as per local standard, for this trial that will enroll women who meet the criteria of being non-childbearing. Redundant text deleted from Table 1 Footnote 10.	Section 8.2.10 Pregnancy Testing

Change	Rationale	Affected Section(s)
Safety, Adverse Events, and Study Oversight		
Clarification of follow-up for AEs. Added text: "*AEs will be followed through to event resolution or stability or death.*"	Clarification of language.	Section 9 Adverse Events
Section 9.11.1 Safety Review Committee addition of text regarding additional consultation The SRC may be consulted on other aspects of the study conduct, as applicable.	Addition of text for consultation with SRC regarding eligibility when data are not clearly interpretable.	Section 1.1 Protocol Synopsis; Section 9.11.1 Safety Review Committee
Stopping Rule		
Section 10.1 clarification of stopping rule 3. Original: "3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5." Will be modified to: "3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5 *persisting for >2 weeks*".	Addition of a time limit on the allowable duration of elevated ALT, AST, or total bilirubin to avoid unnecessary stoppages in the study.	Section 10.1 Stopping Rules for the Study
General Change		
All relevant laboratory measurement values will also be expressed in the International System of Units (SI) units.	For operational consistency.	Relevant sections throughout the protocol including Inclusion/ Exclusion criteria, as applicable.
All instances of "participant" were changed to "subject" and all instances of "cohort" were changed to "dose level". [redacted] was a typo and was corrected to [redacted] throughout. Minor editorial and formatting changes throughout as applicable. Section numbering was updated based on additions and deletions. Updated protocol version and date and Medical Monitor signature.		

ACR: albumin to creatinine ratio; AE: adverse event; AESI: adverse event of special interest; ALT: alanine aminotransferase; ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; aPTT: activated partial thromboplastin time; ASCVD: atherosclerotic cardiovascular disease: AST: aspartate aminotransferase; BUN: blood urea nitrogen; CVD: cardiovascular disease; D or d: day; DHA: docosahexaenoic acid; DL: Dose Level; DLT: dose limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; EL: endothelial lipase; EOS: end of study; EPA: eicosapentaenoic acid; FFA: free fatty acids; HbA1c: glycosylated hemoglobin; HBV: hepatitis B virus; HCV: hepatitis C virus; HDL-C: high-density lipoprotein cholesterol; HIV: human immunodeficiency virus; HRT: hormone replacement therapy; INR: international normalized ratio; LDL-C: low-density lipoprotein cholesterol; LTFU: long-term follow-up; LVEF: left ventricular ejection fraction; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI: magnetic resonance imaging; NAT: nucleic acid testing; OBD: optimum biological dose; PCSK9: proprotein convertase subtilisin/kexin type 9; PDFF: protein density fat

fraction; PET: positron emission tomography; PK: pharmacokinetic; PT: prothrombin time; PTT: partial thromboplastin time; rMPI: radionuclide myocardial perfusion imaging; RNA: ribonucleic acid; RP2D: recommended Phase 2 dose; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; SI: International System of Units; SPECT: single-photon emission computed tomography; SRC: Safety Review Committee; SSRI: selective serotonin reuptake inhibitor; TEAE; treatment-emergent adverse event; TG; triglyceride(s); ULN: upper limit of normal; VLDL: very low-density lipoprotein.

14.4.5. Summary of Changes to Protocol V2.0, Amendment 1

Protocol CRSP-CVD-400 Version 2.0 (Amendment 1), dated 05 May 2023, was internally approved per the sponsor's standard operating procedure. However, Version 1.0 dated 27 January 2023 was not submitted to a health authority, Institutional Review Board, or Ethics Committee and was revised to Version 2.0, dated 05 May 2023, to reflect changes to the study conduct based on the sponsor's decision.

14.4.6. Protocol History

Document/Version	Date	Global/Country/Site Specific
Original Protocol/Version 1.0	27 January 2023	Global
Protocol Amendment 1/Version 2.0	05 May 2023	Global
Protocol Amendment 2/Version 3.0	08 December 2023	Global
Protocol Amendment 3/Version 4.0	12 January 2024	Global
Protocol Amendment 4/Version 5.0	03 June 2024	Global
Protocol Amendment 5/Version 6.0	18 April 2025	Global

15. APPENDIX: GLOSSARY OF TERMS

List of Abbreviations

Abbreviation	Term
ACC	American College of Cardiology
ADA	anti-drug antibody
AE	adverse event
AESI	adverse event of special interest
AHA	American Heart Association
ALT	alanine aminotransferase
ANGPTL3	angiopoietin-like 3
AP	alkaline phosphatase
ApoB	apolipoprotein B
ASCVD	atherosclerotic cardiovascular disease
AST	aspartate aminotransferase
BMI	body mass index
Cas9	CRISPR-associated protein 9
CCS	Canadian Cardiovascular Society
CNS	central nervous system
CRF	case report form
CRISPR	clustered regularly interspaced short palindromic repeats
crRNA	crispr RNA
CTCAE	Common Terminology Criteria for Adverse Events
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DL	Dose Level
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DP	drug product
DS	drug substance
DSB	double-stranded break
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form

Abbreviation	Term
EL	endothelial lipase
eLBW	estimated lean body weight
EOS	end of study
EPA	eicosapentaenoic acid
FAS	full analysis set
FCS	familial chylomicronemia syndrome
FDA	Food and Drug Administration
FFA	free fatty acids
FIH	first-in-human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GOF	gain-of-function
GP1HBP1	glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1
HDL-C	high-density lipoprotein cholesterol
HDR	homology directed repair
HeFH	heterozygous familial hypercholesterolemia
HIV	human immunodeficiency virus
HoFH	homozygous familial hypercholesterolemia
HRT	hormone replacement therapy
HTG	hypertriglyceridemia
ICF	informed consent form
ICH	International Conference on Harmonisation
IDL	intermediate-density lipoprotein
IgM	immunoglobulin M
indel	insertion or deletion
INR	international normalized ratio
IRB	Institutional Review Board
IRR	infusion-related reaction
IV	intravenous
kPa	kilopascals
LDL-C	low-density lipoprotein cholesterol
LDLR	low-density lipoprotein receptor gene
LFT	liver function test

Abbreviation	Term
LNP	lipid nanoparticle
LOF	loss-of-function
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase
LTFU	long-term follow-up
LVEF	left ventricular ejection fraction
MCS	multifactorial chylomicronemia syndrome
MRE	magnetic resonance elastography
MRI-PDFF	magnetic resonance imaging-protein density fat fraction
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
NAT	nucleic acid testing
NHEJ	nonhomologous end joining
NHP	non-human primate
NOAEL	no-observed-adverse-effect level
OBD	optimal biological dose
PAM	protospacer adjacent motif
PCSK9	proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamic(s)
PDFF	protein density fat fraction
PK	pharmacokinetic(s)
PT	prothrombin time
RNA	ribonucleic acid
RP2D	recommended Phase 2 dose
SAE	serious adverse event
sgRNA	single-guide RNA
SpCas9	*Streptococcus pyogenes* CRISPR-associated protein 9
SRC	Safety Review Committee
SSRI	selective serotonin reuptake inhibitor
TEAE	treatment-emergent adverse event
TG	triglyceride(s)
tracrRNA	trans-activating crispr RNA
ULN	upper limit of normal

Abbreviation	Term
VLDL	very low-density lipoprotein

16. REFERENCES

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Signature Page

Workflow Step: Approval	Name: [redacted] Signature Capacity: Clinical Development Date: 18-Apr-2025 18:11:05 GMT+0000

E-signatures are binding of traditional handwritten signatures. E-record data is stored in CRISPR's validated DMS and compliant with regional requirements.

CLINICAL STUDY PROTOCOL

CRSP-CVD-400

A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias

Study Drug: CTX310
Study Phase: 1
Date of Original Protocol: 27 January 2023

Date of Protocol Amendment: 18 April 2025, Amendment 5
Version: 6.1

Sponsor Emergency Contact	[redacted]	[redacted]

CRISPR Therapeutics AG
Baarerstrasse 14
CH 6300 Zug, Switzerland

Confidentiality Statement

SIGNATURE PAGES

PROTOCOL APPROVAL SIGNATURE PAGE

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	18 April 2025
Version	6.1
Amendment	5

Reviewed and approved by:

{see electronic signature page at the end of the document.}

[redacted]
Medical Monitor
CRISPR Therapeutics AG

Date

PROTOCOL ACCEPTANCE FORM

Protocol	CRSP-CVD-400
Title	A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias
Date	18 April 2025
Version	6.1
Amendment	5

I have carefully read this protocol and agree that it contains all of the necessary information required to conduct this study. I agree to conduct this study as described and according to the Declaration of Helsinki, International Conference on Harmonisation Guidelines for Good Clinical Practice, and all applicable regulatory requirements.

Investigator's Signature Date

Name (printed)

AMENDMENT DETAILS

History of Amendments

This is a substantial amendment Version 6.1 (18 April 2025) to Protocol CRSP-CVD-400 Version 5.1 (17 January 2025), which was in response to the request received from Medicines & Healthcare Products Regulatory Agency (MHRA). Four prior global amendments have occurred. The summary of all amendments is provided in Section 14.4.2, Section 14.4.4, Section 14.4.5, and Section 14.4.6. The protocol history is documented in Section 14.4.7.

Summary of Key Changes to the Country Specific Protocol Version 6.1, Amendment 5 (UK)

This is a substantial amendment to the protocol. The primary reason for this amendment is:

- Replacement of the confirmatory cohort from Phase 1a with Phase 1b disease-specific cohort expansion using a flat dose within a recommended range approved by the Safety Review Committee (SRC) following evaluation of the totality of pharmacokinetic (PK)/pharmacodynamic (PD) and safety data from dose escalation based on estimated lean body weight in mg/kg of CTX310.
- Further clarified the study population as follows (in alignment with MHRA feedback incorporated in the prior protocol version [V5.1_Amendment 4] submitted):
  - Severe HTG with history of ASCVD (secondary prevention only)
  - HoFH (primary or secondary prevention of ASCVD)
  - HeFH (primary or secondary prevention of ASCVD)
  - Mixed hyperlipidemias with history of ASCVD (secondary prevention only)
- Addition of a disease-specific exploratory objective/endpoint
- Addition of disease specific inclusion/exclusion criteria
- Update of pre-infusion prophylaxis regimen

This amendment is not likely to have an impact on safety or rights of the study subjects, or on the reliability and robustness of the data generated in the clinical trial.

All key changes made in Version 6.1, Amendment 5 for the UK are tabulated as Summary of Changes in the Current Amendment in Section 14.4.1.

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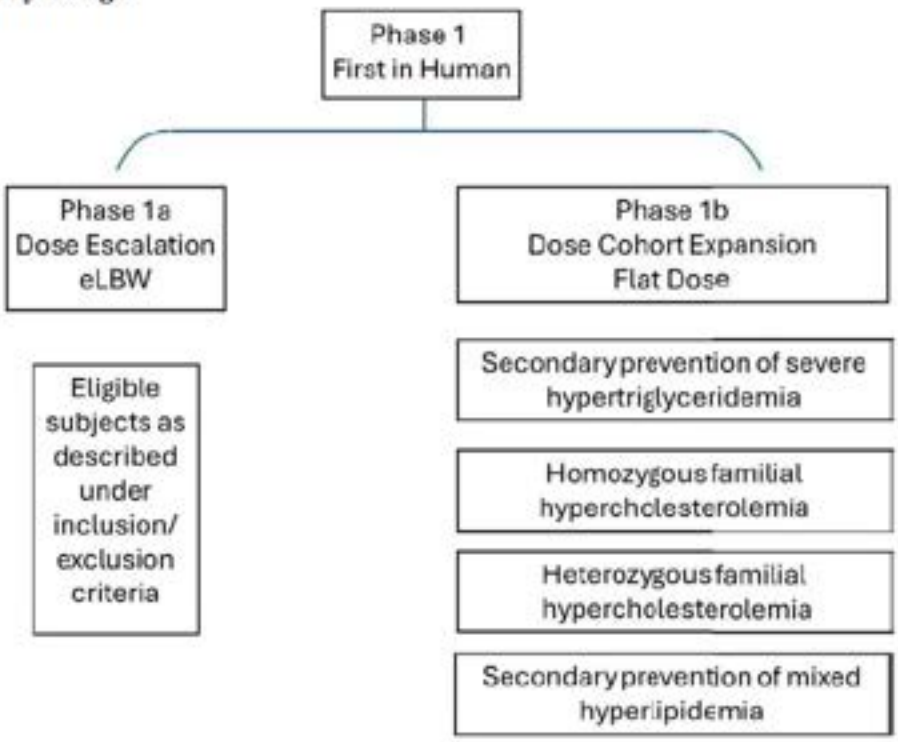
1. PROTOCOL SUMMARY

1.1. Protocol Synopsis

Sponsor: CRISPR Therapeutics AG	**Protocol Number**: CRSP-CVD-400
Name of Investigational Product: CTX310	**Phase of Development**: 1
Protocol Title: A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR-Guide RNA-Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias	
Number of Subjects: Approximately 69 Phase 1a Dose Escalation: Approximately 21 Phase 1b Disease-specific Cohort Expansion: Approximately 48	
Investigators: Multicenter	**Study Type:** Interventional
Investigational Product Description CTX310 is a lipid nanoparticle (LNP) formulation of clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein 9 (Cas9) components for in vivo editing of the target gene angiopoietin-like 3 (*ANGPTL3*). The investigational drug product consists of a capped and polyadenylated spacer Cas9 messenger ribonucleic acid containing N1-methylpseudouridine and a 100 nucleotide–long single-guide ribonucleic acid targeting the gene of interest. CTX310 is designed to utilize CRISPR-Cas9 to disrupt exon 1 of human *ANGPTL3* in the liver, leading to a decrease of ANGPTL3 protein levels.	
Mode of Administration: Subjects will receive CTX310 via intravenous (IV) infusion.	
Study Population The study population will consist of subjects 18 to 75 years (inclusive) who have dyslipidemias with persistently high levels of low-density lipoprotein cholesterol (LDL-C) or triglycerides, above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite diet, lifestyle modifications, and maximum tolerated doses (MTDs) (refractory population), or intolerance to statins.	

Figure S1 Study Design



eLBW: estimated lean body weight

The following dyslipidemias of monogenic, polygenic or undetermined etiology, that are refractory to available treatments and encompass HTG and/or hypercholesterolemia syndromes will be enrolled into the study:

- Severe HTG with history of ASCVD (secondary prevention only)
- HoFH (may be enrolled for primary or secondary prevention of ASCVD)
- HeFH (may be enrolled for primary or secondary prevention of ASCVD)

Mixed hyperlipidemias with history of ASCVD (secondary prevention only)

Populations with atherosclerotic cardiovascular disease (ASCVD) are intended to address secondary prevention with history of prior myocardial infarction (MI) or stroke or known subclinical atherosclerosis on computed tomography (CT) calcium score (>300), CT coronary arteries demonstrating obstructive coronary artery disease (CAD) or coronary angiography demonstrating obstructive CAD.

Following dose escalation (phase 1a), phase 1b will enroll disease-specific cohort expansion of the combined dose escalation populations.

Duration of Participation: All subjects will be monitored for safety, tolerability, pharmacokinetic (PK), pharmacodynamic (PD) and lipid lowering effects for 12 months post infusion in the study. All subjects will be asked to consent to participate in a separate long-term follow-up (LTFU) study for up to 15 years post infusion.

Objectives and Endpoints

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias and to determine the RP2D.	• Incidence of DLTs and frequency of AEs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non-HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To further characterize the safety of CTX310	• Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline

AE: adverse event; AESI: adverse event of special interest; [redacted]

ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; DLT: dose-limiting toxicity; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; PD: pharmacodynamic; PK: pharmacokinetic; RP2D: recommended Phase 2 dose; TEAE: treatment-emergent adverse event; TG: triglyceride(s).

Study Design:

This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll approximately 69 subjects 18 to 75 years (inclusive) with dyslipidemias and increased levels of LDL-C or triglycerides with ASCVD that are refractory or intolerant to statin treatment.

Phase 1a - Dose Escalation

Three to 6 subjects will be enrolled in each of the dose levels (DLs): 0.1, 0.3, 0.6, 0.8, 1.0 and 1.2 mg/kg estimated lean body weight (eLBW) of total ribonucleic acid (RNA) in the LNP formulation. Each subject will receive a single IV dose of CTX310 and will be hospitalized for a minimum of 24 hours after CTX310 infusion (or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment) and will be closely monitored post-infusion for adverse events (AEs) defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All subjects will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Details of the toxicity management guidelines are provided in the protocol.

When the DLT evaluation period ends for the last subject enrolled at each escalation DL, the Safety Review Committee (SRC) will review PK/PD and safety data and will be responsible for making decisions regarding dose escalation or de-escalation.

Phase 1b – Disease-specific Cohort Expansion

Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D). The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.

Following completion of eLBW-based dose escalation, the SRC will review the totality of safety and clinical activity data to delineate a FD for Phase 1b or anticipated RP2D. The FD will be applied to the Phase 1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and LDL) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease-specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. An additional FD may be evaluated in each expansion cohort if the safety or activity profile of the first FD is inadequate and will not exceed the equivalent highest mg dose evaluated during Phase 1a. Administration of CTX310 in Phase 1b will occur in the same manner as in Phase 1a. Phase 1b will confirm the RP2D. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 during dose escalation may be eligible for a second dose based on predefined criteria provided in a future amendment or separate study protocol.

The SRC will be responsible for endorsing the RP2D.

After CTX310 infusion, subjects will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on *ANGPTL3* expression and lipid profile. All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months and those who complete the study, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion.

At each phase 1a DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the SRC before proceeding to the next DL.

Each subject will undergo the following stages:

1. Screening: up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All subjects will be monitored for safety, tolerability, PK, and PD effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: Roll over to a separate LTFU study for up to 15 years post-infusion.

Study Oversight

Safety Review Committee

An SRC consisting of investigators and sponsor representatives will review all available safety data when the DLT observation period ends for the last subject enrolled in each Phase 1a DL and will be responsible for making decisions regarding dose escalation or de-escalation. Throughout dose escalation, for cases in which a dose had been cleared in a DL and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to enroll an additional number of subjects for a total of up to 6 subjects at the current DL to gather additional safety data. The SRC will continue to meet regularly during the dose escalation phase to discuss toxicity management algorithms and to review individual subject cases. Following discussion with the SRC, the sponsor may consult

with the independent Data Safety Monitoring Board (DSMB) regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema.

Following completion of dose escalation and analysis of PK/PD, key lipid, and safety data in Phase 1a, the SRC will evaluate the totality of clinical data to approve a FD for evaluation in Phase 1b expansion cohorts.

The SRC may be consulted on other aspects of the study conduct, as applicable.

Independent Data Safety Monitoring Board

An independent DSMB consisting of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise will be formed at the start of the study, and roles and responsibilities will be described in the DSMB charter. Throughout the study the DSMB will review safety data from Phase 1a dose escalation and Phase 1b disease-specific cohort expansion. The sponsor or designee will be responsible for alerting the DSMB regarding any suspected, unexpected, serious adverse reaction related to CTX310.

Any revisions to the DLT definitions or criteria, dosing schema can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented.

Proposed Starting Dose and Dose Escalation

The CTX310 doses in Table S1 are proposed for evaluation in the 6 planned dose escalation levels in the study, with a minimum of 3 and a maximum of 6 evaluable subjects per DL. The first-in-human (FIH) starting dose is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the non-human primate (NHP) Good Laboratory Practice (GLP) safety and toxicology study (refer to CTX310 Investigator's Brochure).

The initial starting dose of 0.1 mg/kg eLBW of CTX310 refers to the total RNA dose that is based on a NOAEL of 1 mg/kg in the GLP safety and toxicology study. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg lean body weight (LBW). The emerging clinical data on systemically infused LNP-associated therapeutics demonstrates a relatively safe profile. A 3-fold safety factor is proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies. With gradual increments in DLs, dose escalation is expected to proceed from 0.1 to 1.2 mg/kg eLBW. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data (Section 5.5).

Table S1: Phase 1a - Dose Escalation of CTX310

Dose Level*	Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6
4	0.8 [2]
5	1.0 [3]
6	1.2 [4]

DL: Dose Level; eLBW: estimated lean body weight; RNA: ribonucleic acid; SRC: Safety Review Committee.

* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose.

[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW.

[2] Following review of clinical data by the sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.

[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.

[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.

Dosing Within a Dose Level in Phase 1a Dose Escalation

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 subjects will be treated at each DL depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in LFTs observed after dosing with CTX310 resolved within 14 days, the dosing between each subject within a DL will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs) have returned to <2 × baseline or to normal levels, whichever is later. If the safety evaluation of a subject is acceptable, the next subject in the DL may be dosed.

Dose escalation may proceed when all subjects in the preceding DL have completed dosing, the last subject has completed ≥30-day safety evaluation, and the cumulative safety data of all treated subjects at that DL demonstrate an acceptable safety profile, as determined by the SRC.

Rules for Dose-limiting Toxicity Assessment During Phase 1a Dose Escalation

Subjects must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable subject (i.e., a subject that has been administered CTX310 and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable subjects are required per DL during Phase 1a.

An adequate interval will be applied between current lipid-lowering treatments a subject is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities.

Note: Any subject who experiences a DLT will be considered evaluable. Data for all subjects who receive CTX310 will be part of the safety analysis set.

Dose escalation will be performed according to the following rules:

- If 0 of 3 subjects experience a DLT, escalate to the next DL.
- If 1 of 3 subjects experiences a DLT, expand the current DL to 6 subjects.
  - If 1 of 6 subjects experiences a DLT, escalate to the next DL.

- If ≥2 of 6 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL.

- If ≥2 of 3 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 or any optional de-escalation DL as specified in the table above, de-escalate to previous DL or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- No dose escalation beyond highest dose planned or listed for the study (Table S1). Following review of clinical data by the sponsor and SRC, additional intermediate dose levels, besides the prespecified ones in the DL table above, may be added during the course of dose escalation to support the identification of a safe and effective dose.

Phase 1b – Disease-specific Cohort Expansion

Following completion of eLBW-based dose escalation (Table S1), analysis of PK/PD, key lipid and safety data, and approval by the SRC, a safe and efficacious FD will be evaluated in 4 disease-specific cohort expansion cohorts. Each disease-specific cohort may enroll up to 12 subjects.

Following endorsement from the SRC, the sponsor will declare the RP2D based on the totality of clinical data for each disease-specific cohort for future studies. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 during dose escalation may be eligible for a second dose based on predefined criteria provided in a future amendment or separate study protocol.

Toxicities will be graded and documented according to criteria described in the protocol.

All cumulative adverse events (AEs) occurring outside the DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the DSMB.

Dose-limiting Toxicities: Rationale and Criteria

The DLT definitions used in this study are informed by nonclinical studies of CTX310 and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas-9–based genome editing therapy. AEs that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

1. Any CTCAE grade ≥3 elevations in alanine aminotransferase (ALT) and aspartate transaminase (AST) that persist for >14 days and is assessed by the investigator as related to investigational product.
2. Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) that is assessed by the investigator as related to investigational product.
3. Any other CTCAE grade 3 laboratory abnormality that persists ≥7 days and is assessed by the investigator as related to investigational product.
4. Any other CTCAE grade 4 laboratory abnormality that is assessed by the investigator as related to investigational product.
5. Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product.

Study Eligibility

Inclusion Criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

Phase 1a and Phase 1b

1. Age of ≥18 and ≤75 years at the time of signing the informed consent.
2. Able to provide written informed consent.
3. Diagnosis of persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment. Diagnosis of HeFH or HoFH with or without ASCVD. In the Phase 1b cohort expansion part of the study:
    a. Diagnosis of severe HTG defined by elevated fasting TG levels of >500 mg/dL [5.65 mmol/L] in subjects with ASCVD at screening, despite treatment
    b. Diagnosis of mixed hyperlipidemia defined by elevated fasting TG levels of >150 mg/dL – 499 mg/dL (1.7 mmol/L – 5.6 mmol/L) with LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment
4. The lipid levels must be refractory to the MTDs of statins for at least 6 months prior to screening or the subject must be intolerant to statins.
5. Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Subjects on statins must be on a stable dose for 30 days before screening, with no planned dose increase during the study participation.
7. Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation except as required by the protocol.
8. Female subjects must be postmenopausal, defined as:
    - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
    - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male subjects must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in the protocol, from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.

Exclusion Criteria

To be eligible for entry into the study, the subject must not meet any of the exclusion criteria listed below:

Phase 1a and Phase 1b

1. Subjects with familial chylomicronemia syndrome (FCS).

2. Evidence of liver disease, defined as:
   a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >1.5 × ULN, or
   b. Prothrombin time (international normalized ratio) >1.5 × ULN, or
   c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography.
3. Complete blood count: Neutrophils <1500 cells/μL (<1.5 × 10^9/L); lymphocytes <500 cells/μL (0.5 × 10^9/L); hemoglobin <11 g/dL (110 g/L) for males, <10 g/dL (100 g/L) for females; or platelet count <100,000/μL (100 × 10^9/L).
4. Estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick or urine albumin to creatinine ratio of >300 mg/g.
6. Inadequate diabetes control, with glycosylated hemoglobin (HbA1c) ≥8%.
7. History of alcohol or substance use disorder.
8. History of a significant coagulation disorder.
9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.5 and >5.0 mIU/L).
10. Cardiac left ventricular ejection fraction <50% by echocardiogram.
11. Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm^2).
12. Peripheral pulse oximetry saturation of <90%.
13. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic of >90 mmHg, confirmed by a repeat measurement.
14. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
15. Acute coronary syndrome event within 24 weeks prior to Day 1.
16. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
17. Acute pancreatitis within 12 weeks prior to Day 1.
18. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.

23. Positive serology for human immunodeficiency virus type 1 or type 2, hepatitis B virus (hepatitis B core antibody testing or hepatitis surface antigen or nucleic acid testing [NAT]), or hepatitis C virus (hepatitis C antibody testing or NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior malignancy within the past 5 years, or current malignancy (except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ and carcinoma in situ of the cervix or breast), or myeloproliferative disorder, or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, have an intact uterus and at least 1 ovary, and are less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
28. Administration of vaccines 30 days before CTX310 infusion.

For Phase 1b only

29. Treatment with evinacumab within 20 weeks prior to Day 1.

Statistical Methods

Sample Size

The study will enroll approximately 69 subjects to provide a preliminary evaluation of safety and efficacy of CTX310.

Analyses

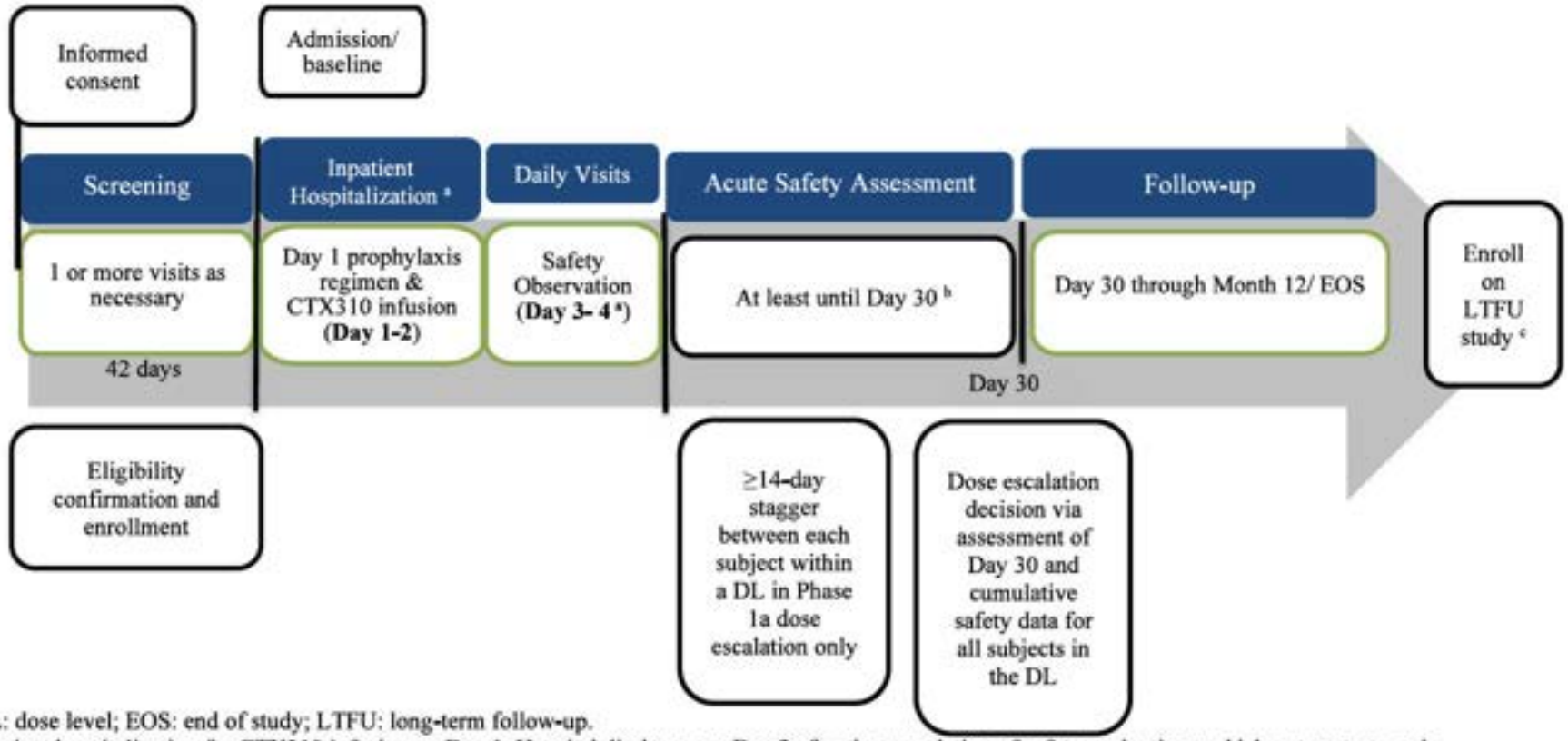
The analysis sets are described in the statistical analyses section of the protocol.

The safety and tolerability of CTX310 will be assessed in the safety analysis set using descriptive summaries. Summaries of AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each DL of CTX310 and overall. Summaries of AEs will focus on treatment-emergent adverse events (TEAEs). The incidence of TEAEs will be summarized by system organ class and preferred term, protocol-specified severity grade, and relation to CTX310. The incidence of DLTs, serious adverse events (SAEs), and AESIs will be also summarized. Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of clinically significant laboratory abnormalities and other clinically significant safety measure abnormalities (e.g., ECG) will be summarized.

The preliminary efficacy of CTX310 will be assessed in the full analysis set using descriptive summaries. The percentage changes in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each CTX310 DL. Categorical summaries based on appropriate cutoff at selected time points, including 26 and 52 weeks after infusion, may be provided.

PK and PD data will be assessed descriptively and by exploratory modeling, as applicable.

1.2. Study Schema



DL: dose level; EOS: end of study; LTFU: long-term follow-up.

[a] Inpatient hospitalization for CTX310 infusion on Day 1. Hospital discharge on Day 2 after the completion of safety evaluation and laboratory tests and a minimum of 24 hours after the completion of the CTX310 infusion. Daily safety visits on Day 3 and 4. Inpatient hospitalization may be extended or following hospital discharge the subject may be readmitted or additional daily safety visits beyond Day 4 may be required at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

[b] For each DL during Phase 1a dose escalation, there will be a safety monitoring period of ≥14 days between the treatment of each subject and any subsequent subjects within the DL, or until the subject is clinically stable and all laboratory values (including liver function tests) have returned to <2 × baseline or to normal levels, whichever is later.

[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for roll over into a separate LTFU study for up to 15 years post-infusion

1.3. Schedule of Assessments

Table 1: Schedule of Assessments

		Treatment	Follow-up											
	Screening[1]	Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Eligibility and Other Assessments														
Informed consent	X													
Demographics, medical history	X													
History of pancreatitis events (for subjects with severe HTG)[4]	X													
Physical exam[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height, weight, BMI, waist to hip ratio	X													X
Vital signs[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver MRE or FibroScan[7]	X													
Liver MRI-PDFF or ultrasound[7]	X													X
Echocardiogram [8]	X[7]													
12-lead ECG[9]	X	X	X			X			X		X	X		X
Eligibility confirmation[10]	X													

Assessment	Screening[1]	Treatment	Follow-up											
		Inpatient Hospitalization[2]		Daily Safety Visits										
	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Treatment														
Pre-infusion prophylaxis regimen[11]	X	X												
CTX310 infusion[12]		X												
Safety Assessments														
Acute DLT assessment		X	X	X	X	X	X	X	X					
Adverse events	X													
Concomitant meds	X													
Laboratory Assessment (Local)[13]														
Serum chemistry	X	X	X	X	X	X	X	X	X		X	X	X	X
Urinalysis	X		X		X				X		X	X	X	X
Coagulation panel	X	X	X	X	X	X	X	X	X		X	X	X	X
Pregnancy test[14]	X													X
Hematology (CBC)	X	X	X	X	X	X	X		X			X		X
HbA1c	X											X	X	X
Thyroid function	X											X		X
eGFR (MDRD equation)	X								X			X		X
Viral serology	X													

		Treatment	Follow-up											
	Screening[1]	Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Cardiac biomarker test[13]		X				X			X		X			
Laboratory Assessments (Central)														
Genetic testing[15]	X													
Lipid panel[16]	X						X		X	X	X	X	X	X
Biomarkers (Plasma, Central)[17]														
ANGPTL3 levels[18]	X						X		X		X	X	X	X
PK studies [19]	X	X	X	X	X	X	X		X		X	X		X
Exploratory Biomarkers (Central)														
Immunogenicity[20]	X					X			X		X	X		X
Whole blood for storage [21]	X													
Plasma for storage[22]	X													
Serum[23]	X	X	X	X										

AESI: adverse event of special interest; ANGPTL3: angiopoietin-like 3; BMI: body mass index; Cas9: CRISPR-associated protein; CBC: complete blood count; CRISPR: clustered regularly interspaced short palindromic repeats; D or d: day; DLT: dose-limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; eLBW: estimated lean body weight; EOS: end of study; HbA1c: glycosylated hemoglobin; HTG: hypertriglyceridemia; ICF: informed Consent Form; LTFU: long-term follow-up; M: month; meds: medications; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI-PDFF: magnetic resonance imaging–protein density fat fraction; PK: pharmacokinetic; W: week.

[1] See Section 8.1.1.1 for detailed guidance. For the subject's convenience, if an assessment was performed before signing the ICF as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe. Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to

determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

[2] Subjects will be hospitalized on Day 1 for CTX310 infusion. Hospital discharge will occur a minimum of 24 hours post completion of CTX310 infusion and after Day 2 safety evaluations are complete and relevant laboratory tests have been reviewed. Subjects must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours completion of CTX310 infusion, or following hospital discharge, the subject may be readmitted or required to stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice. See Section 6.1.2 for discharge criteria and Section 8.1.1 for further description of study periods.

[3] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion (Section 8.1.1.5).

[4] Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment and during the screening period will be collected.

[5] Complete physical exam required at screening, D1, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3).

[6] Vital signs: Blood pressure, heart rate, respiratory rate, oxygen saturation, temperature (Section 8.2.4). Subjects excluded from the study due to uncontrolled hypertension must have blood pressure measurements repeated at least 15 minutes later for confirmation. On Day 1 vital signs should be recorded at the following time points: prior to pre-infusion prophylaxis, prior to the infusion of CTX310, every 15 (± 5) minutes during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital.

[7] Liver imaging: The same type of imaging should be used across all study visits. See Section 8.2.5.

[8] Transthoracic echocardiogram will be performed at screening. See Section 8.2.6 for details.

[9] Electrocardiogram: See Section 8.2.7. Day 1 ECG can be collected within 24 hours prior to Day 1 pre-infusion prophylaxis regimen.

[10] Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment. Also See Laboratory Assessments for Eligibility Confirmation.

[11] Pre-infusion prophylaxis regimen: See Section 6.1.1.

[12] See Section 14.1 for eLBW calculation. See Section 6.1 for other details regarding CTX310 administration.

[13] See listings of laboratory assessments (Table 3 and Table 4 for details). Day 1 local laboratory assessments should be performed within 24 hours prior to the infusion of CTX310. Prior to administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to monitor AESI (Section 9.3 and stopping rules (Section 10.2).

[14] At screening, all females must have a negative serum pregnancy test performed as per local standard. Serum or urine pregnancy test can be performed at EOS/M12 Visit. See Section 8.2.8, Table 3, and Section 8.2.9.

[15] The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.8 and Table 4 for details. Collection of a sample for genetic testing should not be repeated during rescreening, if applicable.

[16] Lipid panel: See Section 8.2.8, Table 4, for listing of lipid panel components. Subjects on apheresis should have lipid levels sampled within 5 days prior to the procedure (pre-apheresis sample on the day of apheresis is also adequate). All lipid panels must be performed after a minimum 8 hour fast.

[17] Sponsor may request discontinuation of sample collections. Continue sample collection for all listed time points until instructed otherwise by the sponsor.

[18] Plasma samples will be obtained to assess ANGPTL3 levels (Section 8.4.2).

[19] PK testing: See Section 8.4.1: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (± 15 min) hours after completion of CTX310 infusion. On D2, D3, and D4 (24 [± 2], 48 [± 2], and 72 [± 2] hours post-infusion time points, respectively), a single sample will be collected. A single sample will also be collected for all other scheduled time points.

[20] Immunogenicity: See Section 8.3.

[21] Whole blood collection: Whole blood samples will be obtained at screening and stored (Section 8.4.3.1).

[22] Plasma for storage: See Section 8.4.3.2.

[23] Serum samples for exploratory biomarker assessments (e.g., cytokines): See Section 8.4.3.3: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (±15 min) hours after completion of CTX310 infusion. On D2 and D3 (24 [± 2] and 48 [± 2] hours post-infusion time points, respectively), a single sample will be collected.

2. INTRODUCTION

CTX310 is being developed by CRISPR Therapeutics AG (sponsor) for the treatment of subjects with refractory dyslipidemias with persistently elevated levels of low-density lipoprotein cholesterol (LDL-C) with established atherosclerotic cardiovascular disease (ASCVD). It is an in vivo gene editing therapy designed to target the gene for angiopoietin-like 3 (*ANGPTL3)*.

This first-in-human (FIH) study will evaluate the safety and tolerability of CTX310 in high-risk adult subjects with dyslipidemias refractory to available treatments.

2.1. Dyslipidemias

Dyslipidemias are among the most commonly detected and treated chronic conditions. They are characterized by abnormal levels of related lipoprotein species and abnormal serum levels of cholesterol, TG, or both. One of the most common clinical consequences of dyslipidemias is increased risk of ASCVD, which is associated with elevated levels of non–HDL-C (primarily LDL-C) and TG. With ASCVD remaining the leading cause of death and disability worldwide (Barquera et al., 2015), new treatment options to add to the current standard of care are clearly needed.

The management of dyslipidemias remains the cornerstone of cardiovascular disease (CVD) prevention. As reported by the American Heart Association (AHA) in 2021, 38% of adults (93.9 million) in the United States (US) had total cholesterol levels ≥200 mg/dL (5.2 mmol/L) from 2015 to 2018, with elevated levels of LDL-C (≥130 mg/dL (3.4 mmol/L)) reported in 29% of adults from 2013 to 2016 (Virani et al., 2021a). Dyslipidemias involving elevated levels of LDL-C (hypercholesterolemia), triglyceride(s) (TG, hypertriglyceridemia [HTG]), or both also contribute to CVD and its associated risks, including type 2 diabetes, chronic kidney disease, and nonalcoholic fatty liver disease (Vijayaraghavan, 2010; Yang et al., 2021). Additional clinical consequences associated with rare dyslipidemias such as severe elevations in TG include increased risk of pancreatitis.

Patients with dyslipidemias are typically treated with lipid-lowering therapies, which may include a statins as first line therapy, and addition of ezetimibe, lomitapide, icosapent ethyl, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors (monoclonal antibodies and ribonucleic acid [RNA] inhibitor), and monoclonal antibody targeting ANGPTL3, where indicated and accessible. Despite all available treatments, only 45% of patients achieve target lipid levels suggested by AHA and American College of Cardiology (ACC) guidelines, especially patients who are at very high risk of cardiovascular events (Pearson et al., 2021; Rallidis et al., 2020), with approximately 50% of patients with ASCVD meeting the definition of high risk (An et al., 2020; Sajja et al., 2021).

Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes as described in Section 2.1.1 and Section 2.1.2, respectively.

2.1.1. Hypertriglyceridemia

Per the Endocrine Society Clinical Practice Guidelines and the National Cholesterol Education Program Adult Treatment Panel III, normal fasting TG levels can be defined as <150 mg/dL (1.7 mmol/L), borderline high as 150 to 199 mg/dL (1.7 to 2.3 mmol/L), high as 200 to 499 mg/dL (2.3 to 5.6 mmol/L), and very high or severe as >500 mg/dL (5.65 mmol/L).

Hypertriglyceridemia is separated into 2 populations: secondary causes related to disease, medications, or diet, and primary genetic syndromes or susceptibility (Rygiel, 2018). Elevated TG levels (>150 mg/dL [1.7 mmol/L]) are present in approximately 33% of adults in the US and are most commonly due to secondary causes rather than primary genetic syndromes (Oh and Trivette, 2020).

Acquired causes of HTG are most commonly due to medical conditions (e.g., diabetes mellitus, metabolic syndrome, central obesity, hypothyroidism, chronic kidney disease, autoimmune disorders), medications, and diet/lifestyle (e.g., alcohol use, physical activity) (Rygiel, 2018).

Genetic causes of HTG may be characterized as multifactorial chylomicronemia syndrome (MCS) and familial chylomicronemia syndrome (FCS). MCS is a polygenic condition caused by heterozygous mutations in the lipoprotein lipase gene (*LPL*) or in the genes for apolipoprotein C2 (*APOC2*), apolipoprotein A5 (*APOA5*), lipase maturation factor 1 (*LMF-1*), or glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (*GP1HBP1*), or by multiple variants that are expressed in the presence of secondary factors (D'Erasmo et al., 2019). MCS is associated with a high CVD risk (Sarwar et al., 2007; Virani et al., 2021b) and affects approximately 1 in 250 to 1 in 800 persons (Fan et al., 2020; Laufs et al., 2020; Paquette and Bernard, 2022). TG levels range from the upper level of normal to severe (>150 to 1999 mg/dL [1.7 to 22.6 mmol/L]).

FCS is a rare monogenic condition caused by the same homozygous or compound heterozygous mutations observed in MCS. As most FCS patients are either LPL-deficient or lack sufficient LPL activity, these patients will likely not benefit from ANGPTL3-directed therapies such as CTX310 and are therefore excluded from this study.

The risk of acute pancreatitis is elevated with increasing levels of serum triglycerides. Severe hypertriglyceridemia ≥500 mg/dL is an established risk factor for acute pancreatitis (Gouni-Berthold et al., 2023). Although TG levels close to normal may be preferable, levels <500 mg/dL represents a safe therapeutic target for prevention of recurrences (Scherer et al., 2014).

Given the need for a significant improvement in TG-lowering therapies, based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of circulating and hepatic TGs.

2.1.2. Familial Hypercholesterolemia

Familial hypercholesterolemia is characterized by lifelong elevations in LDL-C and can be separated into 2 forms: homozygous familial hypercholesterolemia (HoFH) and heterozygous familial hypercholesterolemia (HeFH).

HoFH is a rare monogenic autosomal disorder with a prevalence of 1:300,000, and is characterized by significant elevations in LDL-C (Raal et al., 2020). Patients with HoFH may

develop tendon xanthomas (lipid deposits) and can develop premature CVD, including heart attack and aortic valve disease, when they are teenagers or in their 20s. Without aggressive treatment, patients may die before age 30. The majority of HoFH (85% to 90%) is due to a biallelic deficiency or defect in the LDL receptor gene (*LDLR*). The remaining population have a defect in *ApoB* (loss-of-function [LOF] mutation) or *PCSK-9* (gain-of-function [GOF] or the rare autosomal recessive hypercholesterolemia (Sjouke et al., 2015)). LDL-C levels are elevated due to a failure to synthesize LDLR (receptor-negative), or defective binding or release at the lipoprotein receptor interface, resulting in the inability to clear LDL-C from circulation and giving rise to LDL-C levels of >400 mg/dL (10.3 mmol/L). Traditional lipid-lowering therapies, including high-dose statins, PCSK9 inhibitors, bile acid sequestrants, and ezetimibe, have little to modest activity in HoFH. Plasmapheresis or lipoprotein apheresis is used when available. The recent approval of an ANGPTL3-targeting antibody, evinacumab, provides a safe and effective option for patients with HoFH (Raal et al., 2020). When administered at 15 mg/kg on a monthly regimen, study subjects showed a 47% reduction in LDL-C compared to baseline, leaving significant room for improvement for LDL-lowering in this patient population. AHA guidelines recommend LDL-C levels <70 mg/dL (1.8 mmol/L) in patients at high risk for CVD. Compared with repeat dosing of a monoclonal antibody, CTX310 may offer a one-time treatment option for HoFH patients who require additional and or significant LDL-C lowering.

HeFH is a common autosomal dominant disease, affecting approximately 1 in 250 people, with a significantly higher incidence in high-risk ASCVD (1:17) compared to the general population (Sturm et al., 2018). Similar to HoFH, the most common causes of HeFH are pathogenic variants of *LDLR*, which are responsible for 85% to 90% of genetically confirmed HeFH (Benn et al., 2016). Additional pathogenic variants of *ApoB* resulting in decreased binding of LDL to the LDLR, or GOF mutations in *PCSK9* result in increased destruction of LDLR and a >20-fold increase in ASCVD (Soutar and Naoumova, 2007). Although statins lower LDL-C by 18% to 55%, as many as 80% of statin-treated patients with established ASCVD fail to reach guideline-recommended target LDL-C levels (Marz et al., 2018). The addition of ezetimibe and PCSK9 inhibitors such as monoclonal antibodies and RNA inhibitors (inclisiran) to high-dose statin treatment lowers LDL-C by an additional ~15% to 50%, respectively (Grundy et al., 2019; Wright et al., 2021). Patients who are the least responsive to treatment are at highest risk of developing ASCVD. Evinacumab was evaluated in subjects with refractory hypercholesterolemia (LDL-C ≥100 mg/dL [2.6 mmol/L], or LDL-C ≥70 mg/dL [1.8 mmol/L] with ASCVD; (Rosenson et al., 2020). In this Phase 2 study, 70% to 80% of subjects had HeFH, approximately 60% were receiving statin therapy, 100% were receiving a PCSK9 inhibitor, and approximately 30% were receiving ezetimibe, reflecting a population of patients with refractory hypercholesterolemia who may benefit from additional therapies designed to lower LDL-C. A reduction of LDL-C of up to 50% was achieved upon repeat dosing.

Given the need for improvements in TG and LDL-lowering therapies and based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of both LDL-C and TG.

2.2. ANGPTL3

ANGPTL3 regulates plasma lipid levels through inhibition of LPL and endothelial lipase (EL). Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab

exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Pouwer et al., 2020). *ANGPTL3* LOF variants have been associated with decreased levels of TG, LDL-C, non-HDL-C, ApoB, and HDL-C, as well as a 41% lower risk of coronary artery disease (Dewey et al., 2017). Mechanistic studies indicate that ANGPTL3 inhibition leads to clearance of VLDL remnant particles, upstream of LDL formation, and that LDL-C lowering with ANGPTL3 inhibitors is independent of LDL receptor function (Adam et al., 2020; Reeskamp et al., 2021; Wu et al., 2020).

The LOF variants in *ANGPTL3* have been associated with decreased levels of both LDL-C and TG, and a 41% lower risk of coronary artery disease, with no pathologic manifestations despite the presence of low levels of HDL-C (Athyros et al., 2018; Calandra et al., 2017; Dewey et al., 2017; Stitziel et al., 2017; Tarugi et al., 2019).

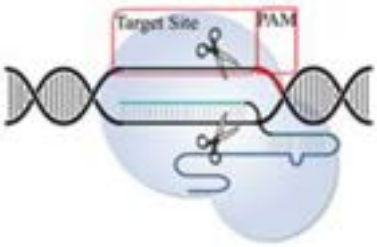
Collectively, these data indicate that *ANGPTL3* is a valid therapeutic target to lower plasma LDL-C, non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who are at high risk of CVD. In vivo disruption of *ANGPTL3* in the liver using CTX310 may therefore provide clinical benefit in the study population with elevated lipid profiles selected for the FIH Phase 1 study.

2.3. CRISPR Technology

CRISPR (clustered regularly interspaced short palindromic repeats) are found flanking foreign deoxyribonucleic acid (DNA) sequences in many bacteria and archaea. CRISPR are an important part of an adaptive bacterial defense system using RNA-guided DNA cleaving enzymes (Barrangou et al., 2007; Hale et al., 2009). The RNAs expressed from CRISPR sequences direct the sequence-specific binding of CRISPR-associated protein 9 (Cas9) nuclease. These key bacterial defense systems were adapted as a programmable RNA-directed CRISPR-Cas9 system for editing genomes (Jinek et al., 2012).

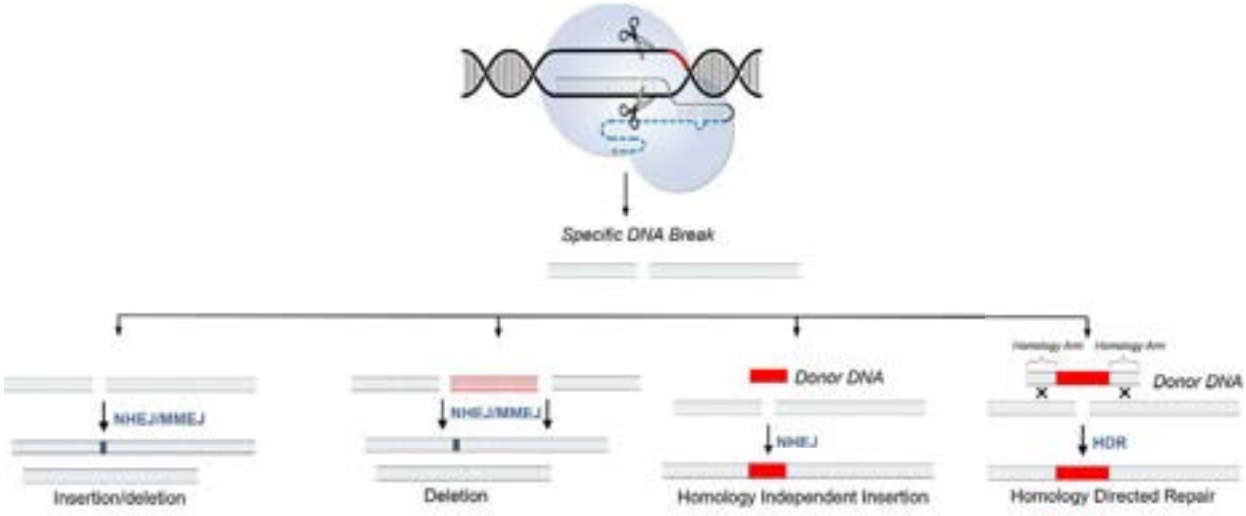
CRISPR-Cas9 systems can be directed by a complex of 2 distinct RNAs: crispr RNA (crRNA) and trans-activating crispr RNA (tracrRNA), or by a single-guide RNA (sgRNA) containing the crRNA and tracrRNA joined by a loop (Jinek et al., 2012). Delivery of Cas9 nuclease and sgRNA into a cell result in cleavage of the cell's genomic DNA at sequences specified by the sgRNA (Figure 1), leaving double-stranded breaks (DSBs). These DSBs are repaired by the cell's own DNA repair machinery. Nonhomologous end joining (NHEJ) is the predominant cellular repair pathway that is active during all phases of the cell cycle (Figure 2). However, NHEJ is an imprecise mechanism for DNA repair and often results in insertions or deletions (indels) at the cut site that can lead to gene disruption and potential LOF. The NHEJ repair pathway can be co-opted to insert DNA sequences at targeted Cas9-sgRNA cut sites in nondividing cells, a process referred to as homology-independent insertion (Figure 2). Homology directed repair (HDR) is the second most common repair mechanism for DSBs but, unlike NHEJ, is only active during late S and G2 phases of the cell cycle. HDR relies on the presence of a homologous repair template (Figure 2) and, as a result, DNA is often repaired faithfully with no indel formation. In cycling cells, HDR in the presence of genomic sequences containing homology arms can be used to correct mutations or introduce novel sequences at specific cut sites.

Figure 1: Schematic of the CRISPR-Cas9 Complex



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; crRNA: crispr RNA; PAM: protospacer adjacent motif; RNA: ribonucleic acid; sgRNA: single-guide RNA; tracrRNA: trans-activating crispr RNA.
CRISPR-Cas9 complex containing a sgRNA wherein the crRNA and tracrRNA are joined by a linker loop.

Figure 2: CRISPR-Cas9–mediated Genome Editing Strategies



Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; DNA: deoxyribonucleic acid; MMEJ: microhomology-mediated end joining; NHEJ: nonhomologous end joining.

Target sites for CRISPR-Cas9 systems are distributed throughout the genome. A requirement is that the target sequence, homologous to the 5' end of the sgRNA and typically 20 nucleotides long, is followed by a protospacer adjacent motif (PAM) sequence (Horvath et al., 2008; Mojica et al., 2009; Shah et al., 2013). For *Streptococcus pyogenes* CRISPR-associated protein 9 (SpCas9), the PAM is any nucleotide followed by a pair of guanines (denoted as NGG).

The Cas9 nuclease searches for the PAM site and adjacent sequence matching the sgRNA 5' end before cleaving the DNA. Target site specificity results from a required combination of the site matching the sgRNA adjacent to a PAM site: the Cas9 nuclease does not bind to sequences without being complexed to a matching sgRNA, and the Cas9 nuclease and sgRNA will not bind or cut unless the target sequence is adjacent to the PAM.

CTX310 utilizes CRISPR-Cas9 to selectively cut exon 1 of *ANGPTL3*, which is specifically expressed in the liver. The resulting indels via NHEJ-mediated repair (active in nondividing

hepatocytes) lead to small frameshift mutations and a premature stop codon, resulting in protein knockdown. The consequence of the gene editing is the reduction of ANGPTL3 protein secreted into circulation.

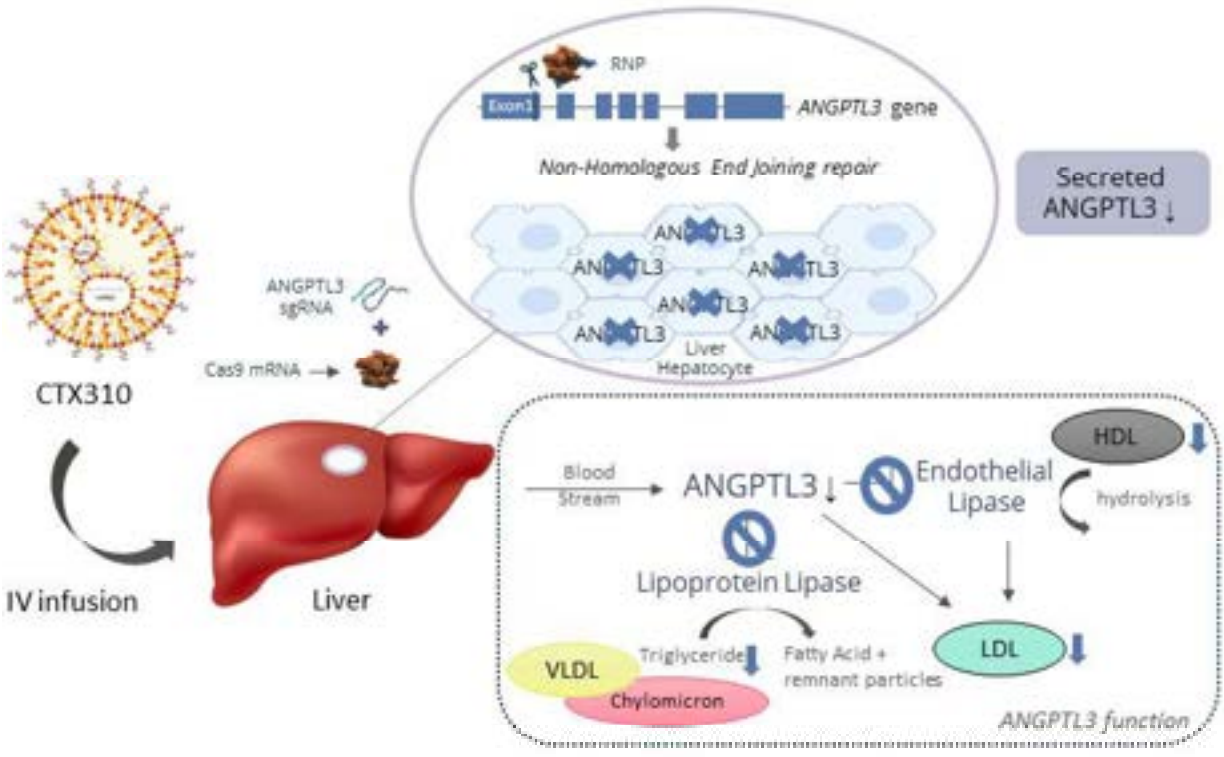
2.4. CTX310

CTX310 is a lipid nanoparticle (LNP) formulation of CRISPR-Cas9 components for in vivo gene editing of the target gene *ANGPTL3*.

The CTX310 drug product (DP) is a sterile formulation that consists of 2 drug substances (DSs): messenger ribonucleic acid (mRNA) encoding SpCas9, and [redacted] sgRNA targeting the gene of interest with a defined mass ratio encapsulated in an LNP, both of which will be individually manufactured prior to coformulation into DP. The DSs and DP will be manufactured and stored according to Good Manufacturing Practice. The first DS is a capped and polyadenylated SpCas9 mRNA containing N1-methylpseudouridine. The second DS, sgRNA, is a 100 nucleotide–long single-stranded oligonucleotide. The DP is an LNP encapsulating the 2 DSs and is composed of 4 lipid components: a cationic lipid, a polyethylene glycol lipid, 1,2-distearoyl-sn-glycero-3-phosphocholine, and cholesterol. The DP LNPs have an average size of ~60 nm and range in size from approximately 50 to 100 nm.

The mechanism of action for CTX310 is the disruption and reduction of the ANGPTL3 biological pathway (Figure 3). Nonclinical data supporting the clinical use of CTX310 are summarized in the Investigator Brochure.

Figure 3: CTX310 Mechanism of Action



ANGPTL3: angiopoietin-like 3; Cas9: CRISPR-associated protein 9; CRISPR: clustered regularly interspaced short palindromic repeats; EL: endothelial lipase; HDL: high-density lipoprotein; IV: intravenous; LDL: low-density lipoprotein; LNP: lipid nanoparticle; LPL: lipoprotein lipase; mRNA: messenger ribonucleic acid; NHEJ: nonhomologous end joining; RNA: ribonucleic acid; RNP: ribonucleoprotein; sgRNA: single-guide RNA; SpCas9: *Streptococcus pyogenes* CRISPR-associated protein 9; TG: triglyceride(s); VLDL: very low-density lipoprotein.

CTX310 is delivered via IV infusion. Similar to other LNPs, CTX310 is expected to be taken up by liver hepatocytes. After uptake, CTX310 escapes from endosomes and releases encapsulated SpCas9 mRNA and sgRNA into cytosol. SpCas9 mRNA is translated to SpCas9 protein, which then forms an RNP complex with sgRNA; this RNP complex shuttles into the nucleus and binds the target sequence. The RNP complex cuts at exon 1 of the *ANGPTL3* locus and introduces frameshift mutations after NHEJ repair. This leads to the knockdown of ANGPTL3 protein expression and reduced secretion from hepatocytes into circulation. The dotted box portrays the role of ANGPTL3 in lipoprotein metabolism. ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; EL hydrolyzes phospholipids, mainly on HDL particles. ANGPTL3 also affects LDL levels through LDL receptor-dependent and -independent (via EL) pathways. Knockdown of ANGPTL3 de-represses the activity of these lipases and leads to reduction of TG and LDL levels.

2.5. Study Rationale

A one-time in vivo disruption of *ANGPTL3* in the liver using CTX310 may provide clinical benefit in patients with dyslipidemia with elevated levels of TG and/or non–HDL-C (including LDL-C) and/or ApoB that are refractory to current treatments, and where compliance to adherence with lifelong medications and optimal lifestyle continue to remain a challenge.

2.5.1. Rationale for Targeting *ANGPTL3*

The sponsor is developing a one-time gene editing therapy for patients with dyslipidemias who have responded inadequately to maximum tolerated doses (MTDs) and adequate duration of currently available treatments and have not achieved the target lipid levels recommended by current guidelines (i.e., who are refractory). The gene editing therapy utilizes CRISPR-Cas9 to specifically target and disrupt *ANGPTL3*, which encodes a regulator of lipoprotein metabolism expressed in the liver (Conklin et al., 1999) and has emerged as a therapeutic target for patients with mixed dyslipidemias. As described in Section 2.2, ANGPTL3 has been shown to inhibit activity of LPL, the main enzyme involved in hydrolysis of TG-rich lipoproteins, and EL, which hydrolyzes HDL phospholipids, and therefore increases TG and other lipids (Kersten, 2021; Shimamura et al., 2007; Shimizugawa et al., 2002). Decreased ANGPTL3 levels have been shown to exhibit higher LPL activity and thus reduced levels of TG (Christopoulou et al., 2019). *ANGPTL3* inhibition can also lead to efficient clearance of VLDL remnant particles via activation of EL in an LDLR-independent mechanism, leading to reduction in LDL-C, non-HDL-C, and ApoB levels (Adam et al., 2020; Rosenson et al., 2020).

Large-scale genetic studies in humans show LOF variants of *ANGPTL3* have low levels of TG and LDL-C and decreased risk of ASCVD (Dewey et al., 2017; Helgadottir et al., 2016) despite low levels of HDL-C (Minicocci et al., 2012; Musunuru et al., 2010; Stitziel et al., 2017). In addition, clinical studies targeting *ANGPTL3* by lowering or inactivating through antisense oligonucleotide or monoclonal antibody treatments have demonstrated efficacy in subjects with various forms of dyslipidemia to markedly reduce plasma LDL-C and TG levels (Graham et al., 2017; Raal et al., 2020; Rosenson et al., 2020; Watts et al., 2019). *ANGPTL3* inhibition also substantially lowers ApoB levels. Mendelian randomization analyses have shown this inhibition has been shown to proportionally decrease risk of CVD (Ference et al., 2019). Together, these studies indicate that *ANGPTL3* is a valid therapeutic target to lower plasma non–HDL-C, ApoB, and TG levels for patients with dyslipidemias who are unable to achieve minimum acceptable target levels of lipids with currently available treatments and who remain at high risk of CVD.

2.5.2. Rationale for the Study Population

Based on the nonclinical understanding of and emerging clinical data from ANGPTL3-directed therapies, and a high unmet need in a treatment-refractory population at high risk for repeat cardiovascular events, this Phase 1 study will include subjects with established ASCVD who have monogenic, polygenic or undetermined etiology, that are refractory to available treatments and encompass HTG and/or hypercholesterolemia syndromes (Section 2.1).

Based on data from non–Good Laboratory Practice (GLP) and GLP toxicology studies in non-human primate (NHP) models in which non-adverse, dose-dependent transient elevations in liver function tests (LFTs) were observed, eligibility criteria for the clinical study will exclude

subjects with underlying liver fibrosis or past infections, alcohol abuse disorder, compromised liver function, or co-morbidities that may compromise liver function. As the liver is the main target organ for CTX310, subjects with coagulation disorders or low platelet counts are also excluded. Subjects who do not meet adequate organ function criteria (e.g., for renal, lung, and cardiac function) are excluded from participating in the Phase 1 study.

As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema based on estimated lean body weight (eLBW), which takes into account gender and height rather than total body weight alone, was selected to reduce the risk of overdosing subjects with higher body mass index (BMI).

Due to the unknown risk of on-target editing in tissues other than the liver, including reproductive organs, women of childbearing potential will be excluded from this study, and male subjects enrolled in the study must agree to use an acceptable method of effective contraception from study consent through 12 months after CTX310 infusion, until additional nonclinical data are available to reassess the eligibility criteria (see Section 4).

The study considers the balance of risk and potential benefit for subjects in this FIH study of CTX310.

2.5.3. Rationale for Lipid Cutoff Values for Inclusion

Per AHA and CCS guidelines (Grundy et al., 2019; Pearson et al., 2021), subjects at high risk of cardiovascular events (i.e., LDL-C ≥70 mg/dL [1.8 mmol/L]) and with established ASCVD are recommended for this study.

The LDL-C levels must be high, in spite of continued use of MTD of statins (as monotherapy or in combination with other lipid lowering agents) for at least 6 months (refractory), to ensure that the high-risk group is included in the study. Subjects who are intolerant to statins constitute another high-risk group due to limited access to other available treatments.

Severe HTG, defined as TG levels of >500 mg/dL (5.65 mmol/L), is a risk factor for ASCVD and is recommended for inclusion in Phase 1b (Gouni-Berthold et al., 2023; Gurevitz et al., 2024). Analyses of the characteristics and prevalence of chronic conditions by TG levels have shown that these conditions and multiorgan disease are common at higher TG level, with a substantially increased risk of pancreatitis associated with this condition (Gouni-Berthold et al., 2023; Gurevitz et al., 2024; Scherer et al., 2014).

2.5.4. Nonclinical Data with CTX310

In NHP studies, a single dose of CTX310 resulted in significant and sustained reductions in TG levels in a dose-dependent manner, and in a mouse *LDLR* knockout model, a mouse surrogate of CTX310 resulted in significant lowering of LDL-C. Together, these nonclinical data, summarized in the Investigator's Brochure, support the use of CTX310 as a one-time treatment to lower atherogenic lipids.

2.5.5. Clinical Data with CTX310

Preliminary data from DL1 (0.1 mg/kg eLBW) and DL2 (0.3 mg/kg eLBW) indicate a favorable safety profile. CTX310 was well-tolerated with no SAEs or serious adverse reactions (SARs),

and no treatment-emergent Grade 3 or higher adverse event (AE). No clinically relevant abnormal coagulation findings, or dose dependent liver enzyme elevations higher than grade 1 were observed (data on file). All treatment-emergent adverse events (TEAEs) were mild to moderate in severity. The benefit-risk profile for CTX310 remains acceptable for continued clinical development.

3. STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias and to determine the RP2D.	• Incidence of DLTs and frequency of AEs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non-HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To further characterize the safety of CTX310	• Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline
Exploratory Objectives	**Exploratory Endpoints**
• To identify changes associated with CTX310 that may indicate or predict clinical response, immunogenicity, safety, or PD activity	• Percentage change in FFA levels over time compared to baseline. • Change in fatty liver disease. • Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required). • For Phase 1b only – Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG.

ADA: anti-drug antibody; AE: adverse event; AESI: adverse event of special interest; [redacted] ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; DLT: dose-limiting toxicity; FFA: free fatty acid; HDL-C: high-density lipoprotein cholesterol; HTG: hypertriglyceridemia; LDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; PD: pharmacodynamic(s); PK: pharmacokinetics; RP2D: recommended Phase 2 dose; TEAE: treatment-emergent adverse event; TG: triglyceride(s).

4. SUBJECT ELIGIBILITY

4.1. Inclusion Criteria

To be considered eligible to participate in this study, a subject must meet all the inclusion criteria listed below:

Phase 1a and Phase 1b

1. Age of ≥18 and ≤75 years at the time of signing informed consent.
2. Able to provide written informed consent.
3. Diagnosis of persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment. Diagnosis of HeFH or HoFH with or without ASCVD. In the Phase 1b cohort expansion part of the study:
    a. Diagnosis of severe HTG defined by elevated fasting TG levels of >500 mg/dL [5.65 mmol/L] in subjects with ASCVD at screening, despite treatment.
    b. Diagnosis of mixed hyperlipidemia defined by elevated fasting TG levels of >150 mg/dL – 499 mg/dL (1.7 mmol/L – 5.6 mmol/L) with LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment.
4. The lipid levels must be refractory to the MTDs of statins for at least 6 months prior to screening or the subject must be intolerant to statins.
5. Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening.
6. Subjects on statins must be on a stable dose for 30 days before screening, with no planned dose increase during the study participation.
7. Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change in frequency during the study participation except as required by the protocol.
8. Female subjects must be postmenopausal, defined as:
    - At least 12 consecutive months of amenorrhea in women with a uterus, without an alternative medical cause; **or**
    - Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).
9. All male subjects must agree to the use of an acceptable method of effective contraception **and** their female partners should also agree to use an effective method of contraception, as defined in Section 14.2, from consent through 12 months after CTX310 infusion.
10. Willing and able to comply with scheduled visits, treatment plan, laboratory tests, contraceptive guidelines, and other study procedures.

4.2. Exclusion Criteria

To be eligible for entry into the study, the subject must not meet any of the exclusion criteria listed below:

Phase 1a and Phase 1b

1. Subjects with chylomicronemia syndromes (e.g., familial chylomicronemia syndrome FCS.
2. Evidence of liver disease, defined as:
    a. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >2 × upper limit of normal (ULN), or total bilirubin value >1.5 × ULN, or
    b. Prothrombin time (international normalized ratio [INR]) >1.5 × ULN, or
    c. Liver elastography measurement of ≥7.5 kPa on FibroScan or liver stiffness measure of >4.15 kPa on magnetic resonance elastography (MRE).
3. Complete blood count: Neutrophils <1500 cells/μL (<1.5 × 10^9/L); lymphocytes <500 cells/μL (0.5 × 10^9/L); hemoglobin <11 g/dL (110 g/L) for males, <10 g/dL (100 g/L) for females; or platelet count <100,000/μL (100 × 10^9/L).
4. Estimated glomerular filtration rate <60 mL/min/1.73 m^2, as measured by Modification of Diet in Renal Disease equation.
5. Diagnosis of nephrotic syndrome or albuminuria >2+ on urine dipstick or urine albumin to creatinine ratio of >300 mg/g.
6. Inadequate diabetes control, with glycosylated hemoglobin (HbA1c) ≥8%.
7. History of alcohol or substance use disorder.
8. History of a significant coagulation disorder.
9. Uncontrolled or untreated thyroid disease (thyroid-stimulating hormone <0.5 and >5.0 mIU/L).
10. Cardiac left ventricular ejection fraction (LVEF) <50% by echocardiogram.
11. Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm^2).
12. Peripheral pulse oximetry saturation of <90%.
13. Uncontrolled hypertension, defined as systolic >160 mmHg and diastolic >90 mmHg confirmed by a repeat measurement.
14. 12-lead electrocardiogram (ECG) findings demonstrating:
    - QTc of >450 ms for males and >470 ms for females at screening.
    - Any other ECG finding deemed clinically significant by the investigator.
15. Acute coronary syndrome event within 24 weeks prior to Day 1.
16. Central nervous system (CNS) stroke within 24 weeks prior to Day 1.
17. Acute pancreatitis within 12 weeks prior to Day 1.

18. Current use or use within 365 days from Day 1 of any hepatocyte-targeted small interfering RNA (except inclisiran).
19. Participation in another clinical study with an investigational drug/product within 30 days of screening or <5 half-lives of the investigational agent, whichever is longer from screening.
20. Current use of chronic systemic corticosteroid therapy, or anabolic agents.
21. Current use of nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
22. Prior treatment with gene therapy/editing product.
23. Positive serology for human immunodeficiency virus (HIV) type 1 or type 2, hepatitis B virus (hepatitis B core antibody or hepatitis B surface antigen or NAT), or hepatitis C virus (hepatitis C antibody testing or NAT).
24. History of any illness or any clinical condition that, in the opinion of the investigator, might confound the results of the study or pose an additional risk in administering study drug to the subject. This may include but is not limited to history of relevant drug allergies, history of CNS disease, history or presence of clinically significant pathology, history of psychiatric illness, or history of familial cancer syndrome.
25. Any prior malignancy within the past 5 years, or current malignancy (except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ and carcinoma in situ of the cervix or breast), or myeloproliferative disorder, or a significant immunodeficiency disorder.
26. Females of childbearing potential (postmenarchal, have an intact uterus and at least 1 ovary, and are less than 1 year since spontaneous amenorrhea) or breastfeeding females.
27. An assessment by the investigator that the subject would not comply with the study procedures outlined in the protocol.
28. Administration of vaccines 30 days before CTX310 infusion.

For Phase 1b only

29. Treatment with evinacumab within 20 weeks prior to Day 1.

5. STUDY DESIGN

5.1. Investigational Plan

This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll of subjects 18 to 75 years (inclusive) who have dyslipidemias with persistently high levels of LDL-C or triglycerides, above the thresholds recommended by ACC and AHA guidelines despite diet, lifestyle modifications, and MTDs (refractory population), or intolerance to statins (see Inclusion Criteria in Section 4.1).

This Phase 1 study will include subjects with monogenic or polygenic refractory dyslipidemias, with ASCVD, that encompass or hypercholesterolemia syndromes, as described in Section 2.5.2. The study is divided into 2 parts (Figure 4): Phase 1a dose escalation followed by Phase 1b disease-specific cohort expansion.

Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D) (Section 5.5). The FD will be a dose associated with optimal biological efficacy, as determined by the intended decrease in ANGPTL3 and key lipid levels with minimum toxicity. Phase1b will confirm the RP2D.

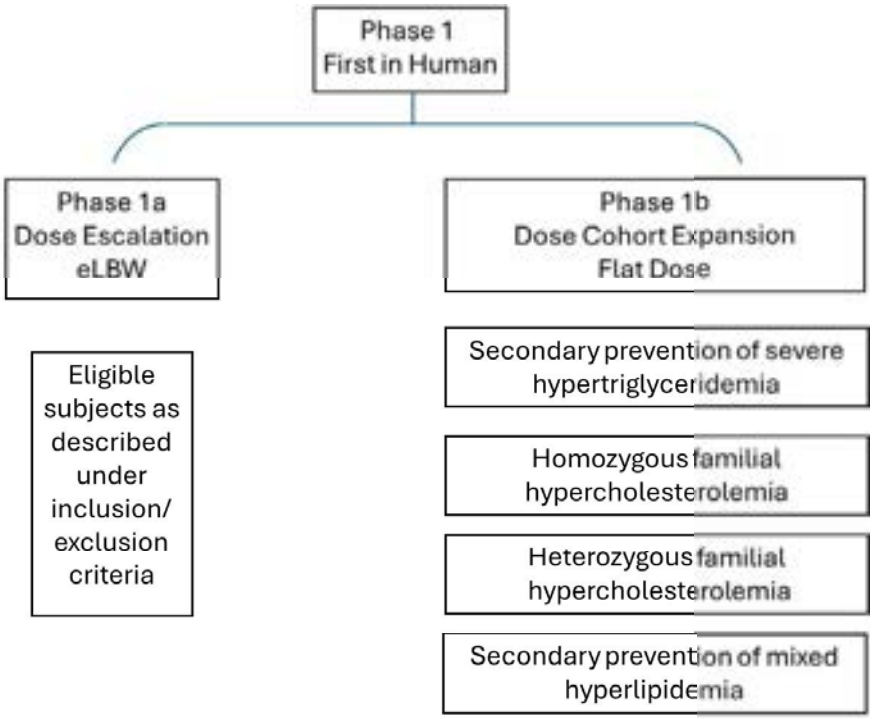
The following dyslipidemias of monogenic, polygenic or undetermined etiology, that are refractory to available treatments and encompass HTG and/or hypercholesterolemia syndromes will be enrolled into the study:

- Severe HTG with history of ASCVD (secondary prevention only)
- HoFH (may be enrolled for primary or secondary prevention of ASCVD)
- HeFH (may be enrolled for primary or secondary prevention of ASCVD)
- Mixed hyperlipidemias with history of ASCVD (secondary prevention only)

Populations with ASCVD are intended to address secondary prevention with history of prior myocardial infarction (MI) or stroke or known subclinical atherosclerosis on computed tomography (CT) calcium score (>300), CT coronary arteries demonstrating obstructive coronary artery disease (CAD) or coronary angiography demonstrating obstructive CAD.

Following dose escalation (Phase 1a), Phase 1b will enroll disease-specific cohort expansion of the combined dose escalation populations.

Figure 4: Study Design



eLBW: estimated lean body weight

In Phase 1a, the majority of subjects enrolled are expected to have HeFH or be of polygenic background due to the high prevalence of HeFH and polygenic hypercholesterolemia (Goldberg and Chait, 2020; McGowan et al., 2019; Sturm et al., 2018). Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing future CVD events in patients with established ASCVD.

Subjects will be asked to continue to take their baseline lipid-lowering medications in the same doses throughout the study; adequate washout periods between the medications and CTX310 infusion have been included, when relevant.

Three to 6 subjects will be enrolled in each of the dose levels (DLs): 0.1, 0.3, 0.6, 0.8, 1.0, and 1.2 mg/kg eLBW of total RNA in the LNP formulation. Within each DL, a minimum 14-day stagger between dosing of CTX310 to each subject is required. Dose escalation will follow the criteria described in Section 5.4.

Following completion of Phase 1a eLBW-based dose escalation, the SRC will review the totality of safety and clinical activity data to delineate a FD for Phase 1b or anticipated RP2D. The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.

The FD will be applied to the Phase1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious

and safe FD which will be evaluated in each of the 4 disease specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase1a. Phase 1b will confirm the RP2D. An additional FD may be evaluated in each expansion cohort if the safety or activity profile of the first FD is inadequate and will not exceed the highest mg dose evaluated during Phase 1a.

Each subject will receive a single intravenous (IV) dose of CTX310 on Day 1 and will be hospitalized for a minimum of 24 hours after completion of CTX310 infusion (or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment). Following hospital discharge, subjects may be readmitted, if needed, for safety assessment. All subjects will be closely monitored post-infusion for adverse events (AEs) defining dose-limiting toxicities (DLTs) during the 30-day acute safety evaluation period. All subjects will receive premedication with a corticosteroid and antihistamines (H1 and H2 blockers) prior to receiving CTX310. Subjects will be required to stay within 1 hour of the infusion site to enable daily (and as needed) clinic visits for safety assessment until completion of Day 4 visit (or longer at the discretion of the investigator for safety assessment).

After CTX310 infusion, subjects will be followed for 12 months with physical exams, regular laboratory evaluations, and assessments for AEs and effects on ANGPTL3 levels and lipid profile. All subjects who receive CTX310, including those who discontinue early and those who complete the study, will be asked to consent to roll over into a separate long-term follow-up (LTFU) study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events.

At each Phase 1a dose escalation DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next DL and to Phase 1b disease-specific cohort expansion. Based on the analysis of the totality of clinical data and the endorsement of the SRC, the sponsor will declare the R2PD.

5.2. Number of Study Subjects

Approximately 69 eligible subjects will be enrolled in the study.

Phase 1a Dose Escalation: Approximately 21 subjects.

Phase 1b Cohort Expansion: Approximately 48 subjects (up to 12 subjects per disease-specific cohort).

5.3. Study Duration

As illustrated in the study schema (Section 1.2), each subject will undergo the following stages:

1. Screening: Up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).

3. Follow-up: All subjects will be monitored for safety, tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: All subjects will be asked to roll over to a separate LTFU study (Section 8.1.1.5).

5.4. Dose Escalation and Disease-specific Cohort Expansion

5.4.1. Dose Escalation Methodology

The CTX310 doses in Table 2 are proposed for evaluation in the 6 planned dose escalation levels in the study, with a minimum of 3 and a maximum of 6 evaluable subjects per DL. The dose rationale based on the nonclinical studies in NHPs is described in Section 5.5.

Table 2: Phase 1a - Dose Escalation of CTX310

Dose Level*	Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW)[1]
1	0.1
2	0.3
3	0.6
4	0.8 [2]
5	1.0 [3]
6	1.2 [4]

DL: Dose Level; eLBW: estimated lean body weight; RNA: ribonucleic acid; SRC: Safety Review Committee
* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose.
[1] Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of single guide RNA + messenger RNA per kg of eLBW. See Section 14.1 for eLBW calculation.
[2] Following review of clinical data by the Sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.
[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.
[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.

Dosing Within a Dose Level in Phase 1a Dose Escalation

Dose escalation will be performed using a standard 3+3 design in which 3 to 6 subjects will be treated at each DL depending on the occurrence of DLTs.

Based on NHP studies in which transient elevations in LFTs observed after dosing with CTX310 resolved within 14 days, the dosing between each subject within a DL will be staggered to evaluate potential toxicities for a minimum of 14 days or until the laboratory values (including LFTs) have returned to <2 × baseline or to normal levels, whichever is later. If the safety evaluation of a subject is acceptable, the next subject in the DL may be dosed.

Dose escalation may proceed when all subjects in the preceding DL have completed dosing, the last subject has completed ≥30-day safety evaluation, and the cumulative safety data of all treated subjects at that DL demonstrates an acceptable safety profile, as determined by the SRC.

Rules for Dose-limiting Toxicity Assessment During Phase 1a Dose Escalation

The SRC will review all available cumulative safety and clinical activity data when the DLT observation period ends for the last subject enrolled in each DL and will be responsible for making dose escalation decisions.

Throughout dose escalation, for cases in which a dose had been cleared in a DL and dose escalation is permitted, the sponsor, in consultation with the SRC, may alternatively decide to enroll an additional number of subjects for a total of up to 6 at the current DL to gather additional safety data.

The sponsor, in conjunction with the investigators and the SRC, will decide whether to classify an event occurring within the first 30 days following infusion as a DLT.

Dose escalation will be performed according to the following rules:

- If 0 of 3 subjects experience a DLT, escalate to the next DL.
- If 1 of 3 subjects experiences a DLT, expand the current DL to 6 subjects.
  - If 1 of 6 subjects experiences a DLT, escalate to the next DL.
  - If ≥2 of 6 subjects experience a DLT in DLs 2, 3, 4, 5 or 6 de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- If ≥2 of 3 subjects experience a DLT in DLs 2, 3, or 4, 5 or 6 any optional de-escalation DL as specified in Table 2, de-escalate to previous DL or declare previous DL the MTD if 6 subjects are already tested at the previous DL.
- No dose escalation is planned beyond the highest DL listed for the study (Table 2). Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones in Table 2, may be added during the course of dose escalation to support the identification of a safe and effective dose.

Toxicities will be graded and documented per criteria described in Section 9.

All cumulative AEs occurring outside the acute DLT evaluation period that are assessed by the investigator and/or sponsor as related to CTX310 will also be discussed with the independent Data Safety Monitoring Board (DSMB).

5.4.2. Optimal Biologic Dose Definition

The OBD is the lowest dose associated with biological efficacy, as determined by intended decrease in ANGPTL3 levels, with minimum toxicity.

5.4.3. Dose-limiting Toxicity Rationale and Definitions

The DLT definitions used in this study are informed by nonclinical studies of CTX310, and published reporting of clinical experience with an LNP-encapsulated, CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021). AEs that have no plausible causal relationship with CTX310 will not be considered DLTs. A DLT will be graded and documented according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. The criteria for assessment of an AE occurring in the first 30 days after dosing for classification as a DLT will include the following:

1. Any CTCAE grade ≥3 elevations in ALT and AST that persists for ≥14 days and is assessed by the investigator as related to investigational product.
2. Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (INR) that is assessed by the investigator as related to investigational product.
3. Any other CTCAE grade 3 laboratory abnormality that persists ≥7 days and is assessed by the investigator as related to investigational product.
4. Any other CTCAE grade 4 laboratory abnormality that is assessed by the investigator as related to investigational product.
5. Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product.

Subjects must receive CTX310 to be evaluated for DLTs. If a DLT-evaluable subject (i.e., a subject that has been administered CTX310 and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. A minimum of 3 evaluable subjects are required per DL during Phase 1a.

An adequate interval will be applied between current lipid-lowering treatments a subject is receiving (i.e., monoclonal antibodies and/or inhibitor RNA therapy) and CTX310 infusion, to avoid overlapping toxicities (Section 6.5.1).

Note: Any subject who experiences a DLT will be considered evaluable. Data for all subjects who receive CTX310 will be part of the safety analysis set.

5.4.4. Phase 1b - Disease-specific Cohort Expansion

Following completion of eLBW-based dose escalation (Table 2), analysis of PK/PD, key lipid and safety data, and approval by the SRC, a FD of CTX310 may be evaluated in a Phase 1b cohort expansion in 4 disease-specific cohorts.

The SRC will endorse the RP2D based on the review of the totality of data including clinical activity and safety of the FD for disease-specific groups. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future protocol amendment or separate study protocol.

5.5. CTX310 Dose Rationale

5.5.1. Dose Escalation in Phase 1a

The clinical study with CTX310 will utilize the same LNP formulation used in the CTX310 nonclinical NHP studies, allowing direct correlations between NHP dose and the expected safety and efficacy in human subjects. As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema is based on eLBW, which takes into account sex and height rather than total body weight alone, and, therefore, was selected to reduce the risk of overdosing obese and overweight subjects.

The FIH starting dose of CTX310 is extrapolated from the no-observed-adverse-effect level (NOAEL) that has been determined in the NHP GLP safety and toxicology study (see Section 2.5.4 and refer to the CTX310 Investigator's Brochure). The initial starting dose of 0.1 mg/kg of CTX310 refers to the total RNA dose that is based on an anticipated NOAEL of 1 mg/kg. A one-third allometric scaling from NHP to human, based on total body surface and application of a safety factor of 3, derives a starting dose of 0.1 mg/kg. The emerging clinical data on systemically infused LNP-associated therapeutics demonstrates a relatively safe profile (Adams et al., 2018; Coelho et al., 2013; Gillmore et al., 2021). A 3-fold safety factor is proposed based on the predicted lack of liver-related AEs in humans based on nonclinical studies (Barros and Gollob, 2012).

Clinical exposure data available to date indicate that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Based on available clinical CTX310 PK from dose levels 1 through 3 (n=9) (data on file), the mean dose normalized $AUC_{0\text{-}Tlast}$ of [redacted] and [redacted] was 3,803,333 ± 1,933,384 and 825,000 ± 241,886 (hr*ng/mL)/(mg/kg), respectively. This exposure is approximately the same as the mean dose normalized $AUC_{0\text{-}Tlast}$ in the pivotal NHP GLP Toxicity study ([redacted]: 3,158,889 ± 990,153; [redacted]: 934,000 ± 30,199 (hr*ng/mL)/(mg/kg); Study 3359-006). This exposure data indicates that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Thus, scaling between NHPs and humans may support a human equivalent NOAEL of 1.0 mg/kg and toleration of 2.0 mg/kg. With gradual increments in DLs, dose escalation is expected to proceed from 0.1 to 1.2 mg/kg. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data.

5.5.2. Disease-specific Cohort Expansion with Flat Dose in Phase 1b

Due to the primary distribution of the LNP-mediated CTX310 delivery to the liver, evaluation of safety and clinical activity of FD of CTX310 is considered appropriate and consistent with other liver-targeted genetic medicines that are administered at a FD (Bakris et al., 2024; Coelho et al., 2023; Desai et al., 2023; Fontana et al., 2024; NCT06128629; Ray et al., 2020; Srivastava et al., 2023; Young et al., 2023). Earlier siRNAs (givosiran, lumasiran) initially relied on weight-based dosing (Balwani et al., 2020; Garrelfs et al., 2021) with little difference in efficacy noted regardless of weight. Similarly, in vivo gene editors that target hepatocytes using LNP vehicles

likewise began development with weight-based dosing (Gillmore et al., 2021), but transitioned to flat-dosing in later phases (NCT06128629) and with newer targets (Longhurst et al., 2024). A similar strategy is planned for Phase 1b, where PK, safety (LFTs) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and LDL) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease-specific cohorts.

6. STUDY TREATMENT

6.1. Administration of CTX310

Subjects will receive a single IV infusion on Day 1, administered under medical supervision during inpatient hospitalization. The date and time of the dose administered will be recorded in the source documents and in the electronic case report form (eCRF).

Infusion of the study drug will be slowed or stopped in the event of an infusion-related reaction (IRR; Section 7.1.1).

Prior to the administration of CTX310 on Day 1, investigator should confirm the ability of the subject to receive the infusion (see Table 1) by ensuring:

- No significant change in clinical status since screening.
- No new, clinically significant findings seen on physical exam, vital signs or ECG.
- No AST, ALT, total bilirubin >2 × ULN and PT (INR) >1.5 × ULN.

Prior to the administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor (see Table 1).

If the infusion is delayed for more than 30 days, the subject will be replaced if deemed necessary for dose escalation decisions.

6.1.1. Pre-infusion Prophylaxis

On Day -1, i.e., the evening prior to CTX310 administration on Day 1, an infusion prophylaxis regimen will be administered to subjects as follows:

- Oral steroid (e.g., dexamethasone approximately 10 mg or equivalent)

On Day 1, within 1 to 2 hours prior to the administration of study drug, an infusion prophylaxis regimen will be administered to subjects as follows:

- IV steroid (e.g., dexamethasone 10 mg or equivalent);
- IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent) or oral H1 blocker (e.g., cetirizine 10 mg or equivalent); and
- IV or oral H2 blocker (e.g., famotidine 20 mg or equivalent).
- Optional oral paracetamol (500 mg to 1000 mg)

6.1.2. CTX310 Post-infusion Monitoring

Following completion of study drug administration, subjects will be observed as inpatients for minimum of 24 hours, or longer if required by local regulation or site practice or at the discretion of the investigator for safety assessment. Subjects must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours post completion of CTX310 infusion, or the subject may be readmitted or required to

stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

Safety and clinical laboratory evaluations, and collection of blood and urine samples will be performed per the schedule of assessment (Table 1). Repeat laboratory evaluations may be required for assessment of AESI/ SAE or to meet study stopping criteria (see Section 9.3 and Section 10.1). All AEs and concomitant medications will be recorded.

Inpatient hospitalization for observation may be extended at the discretion of the investigator to follow and manage AEs as needed.

Subjects will be discharged from the study site when they meet the following criteria:

1. Are clinically stable as per the investigator's judgement.
2. Day 2 safety assessments have been performed and relevant laboratory results have been reviewed.
3. The frequency of monitoring of laboratory AEs can be handled in the outpatient setting (schedule of assessments Table 1 and Section 7.1.2).
4. Subjects are aware of contact information in case of an emergency and agree to remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit.

6.2. Investigational Product Preparation, Handling, Storage, and Accountability

CTX310 DP will be provided as a frozen liquid formulation consisting of 300 mM sucrose in phosphate buffered saline at a target concentration of 2.0 (± 0.4) mg/mL total RNA. CTX310 must be stored frozen at ≤-60°C in a glass vial until time of use. The DP will be stored onsite, thawed, and formulated immediately prior to administration. Refer to the Pharmacy Manual for detailed instructions on preparation, storage, handling, and administration of CTX310.

6.2.1. Investigational Product Accountability

The investigator and sponsor are responsible for accountability and traceability of CTX310 clinical supply.

The investigator will ensure that CTX310 is used in accordance with this protocol and the Pharmacy Manual. Detailed accountability records indicating CTX310 inventory at each clinical site, use by each subject, and disposal will be maintained by the clinical sites. To maintain compliance, the sponsor or its designee will review CTX310 clinical supply accountability records at the clinical sites on an ongoing basis during monitoring visits.

Instructions for destruction of all excess and expired material containing CTX310 will be provided directly by the sponsor. Destruction will be adequately documented and reviewed regularly by the sponsor or its designee and the investigator.

6.3. Comparator Product

This is a single-arm study with no comparator.

6.4. Measures to Minimize Bias: Randomization and Blinding

This is a single-arm, open-label study. Masking is not applicable. Randomization is not used in this study.

6.5. Prior and Concomitant Medications

All medications taken within 30 days before the signing of the informed consent form (ICF) will be recorded. All concurrent therapies, including prescription and nonprescription medications, must be recorded from the date of signed informed consent through 12 months after CTX310 infusion.

6.5.1. Allowed Medications and Procedures

Topical, intra-articular, nasal, inhaled, and ophthalmic steroid therapies are not considered systemic and are allowed. Subjects may continue medications or treatments deemed necessary by the investigators to provide adequate supportive treatment for optimal medical care throughout the study, including previously prescribed medications for management of hypertension and lipid levels except for the prohibited medications listed in Section 6.5.2.

Subjects previously prescribed selective serotonin reuptake inhibitors or hormone replacement therapy are recommended to remain on a stable dose from at least 30 days prior to screening through the end of the study.

The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3 ~~([~~[e.g., ethyl esters of EPA or DHA]~~)~~, bile acid sequestrants, evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study.

The following **dosing windows** surrounding the infusion of CTX310 are suggested for lipid-lowering therapies:

- Monoclonal antibodies (e.g., alirocumab, evolocumab, and evinacumab) may not be administered beginning 7 days before the infusion of CTX310 and until 7 days after Day 1.
- Inclisiran may not be administered beginning 60 days before infusion of CTX310 and until 60 days after Day 1.
- Apheresis procedures may not be performed beginning 14 days before infusion of CTX310 and until 14 days after Day 1.

If a significant lowering of lipids (LDL-C or TG) is observed during the course of the study, i.e., lipid levels decrease to the desired levels (e.g., LDL-C <70 mg/dL [1.8 mmol/L], TG <150 mg/dL [1.7 mmol/L], or non–HDL-C <160 mg/dL [4.1 mmol/L]), adjustments to relevant medications or frequency of apheresis procedures may be instituted by the investigator after discussion with the sponsor (Section 4.1 Inclusion Criterion 7). It is expected that the plan for tapering other lipid-lowering medications or apheresis procedures will be individualized for each subject by the investigator depending on the response to study treatment, underlying genotype, and assessment of risk factors for future cardiovascular events.

6.5.2. Prohibited Medications

Medications prohibited prior to enrolling in the study or administration of CTX310 are noted in the exclusion criteria (Section 4.2) and are as follows:

- Hepatocyte-targeted small interfering RNA or antisense oligonucleotide molecules (except inclisiran).
- Any investigational product.
- Chronic systemic corticosteroids.
- Anabolic agents.
- Nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.
- Prior treatment with a gene therapy/editing product.
- Vaccines should not be administered 30 days before or 30 days after CTX310 infusion.
- All oral anticoagulants (e.g., warfarin, apixaban, rivaroxaban, or dabigatran) should not be administered 30 days before or 30 days after CTX310 infusion.
- Phase 1b only: Evinacumab within 20 weeks prior to Day 1.

6.6. Lifestyle Considerations

Subjects who receive CTX310 must not donate eggs, sperm, blood, or organs for the duration of this study.

For subjects who consume alcohol the following recommendations are provided regarding alcohol intake:

- Abstain from alcohol for 2 weeks before and 2 weeks after CTX310 infusion.
- A maximum of 6 standard drinks per week but no more than 2 standard drinks per day for the duration of the study.

7. SAFETY MONITORING RULES

7.1. General Guidance

Subjects will be closely monitored for DLTs for 30 days after CTX310 infusion. Investigators are required to proactively monitor and treat all AEs in accordance with protocol guidance.

Although this is an FIH study and the clinical safety profile of CTX310 has not been previously described, the following general recommendations are provided based on prior experience with an LNP-formulated CRISPR-Cas9–based genome editing therapy (Gillmore et al., 2021) and *ANGPTL3*-targeted antisense oligonucleotide or interfering RNA therapies (Graham et al., 2017; Watts et al., 2022).

The safety profile of CTX310 will be continually assessed throughout the study, and investigators will be updated on a regular basis with new information regarding the identification and management of potential CTX310-related toxicity (refer to Investigator's Brochure).

7.1.1. Infusion-related Reactions

Infusion-related reactions have been reported with LNP utilizing treatments, occurring in up to 19% of patients receiving patisiran (Moghimi and Simberg, 2022). Infusion-related reactions can be either allergic reactions to foreign particles or non-immune mediated reactions. Most IRRs are mild and typically develop within minutes to several hours of initiation of the drug infusion, although symptoms may be delayed for up to 24 hours. IRRs may affect any organ system in the body. While most reactions are mild in severity, severe or fatal reactions can occur. The most common signs and symptoms of IRR are fever, chills, flushing, itching, alterations in heart rate (including tachycardia) or blood pressure (including hypotension), dyspnea, chest discomfort, back pain or abdominal pain, nausea, vomiting, diarrhea, and various types of skin rashes.

In the event of an acute IRR, the infusion of study drug will be slowed or stopped, and the subject closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study drug administration include but are not limited to acetaminophen/paracetamol, H1/H2 blockers, nonsteroidal anti-inflammatory drugs, epinephrine, supplemental oxygen, IV fluids, and/or corticosteroids. Caution should be exercised in the use of acetaminophen/paracetamol. Delayed IRR (>12 hours) are usually immune-mediated and respond best to corticosteroid treatment (e.g., methylprednisolone).

Following resolution of a mild or moderate IRR that required interruption or slowing of the study drug infusion, administration may resume or continue at the investigator's discretion at a slower infusion rate.

Study drug administration will not be resumed for any subject following a severe IRR until the case is discussed with the medical monitor.

7.1.2. Safety Monitoring Rules for Liver Chemistry Tests

The following rules are adapted from the Food and Drug Administration (FDA) 2009 draft guidance for industry, "Drug-Induced Liver Injury: Premarketing Clinical Evaluation."

Any ALT or AST measurement that is >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) at any time during the study (treatment or post-treatment period), will be reported as AESI (Section 9.3) and the measurement(s) should be confirmed by repeat testing within 24 to 48 hours of all 4 of the usual serum measures (ALT, AST, alkaline phosphatase [AP], and total bilirubin) to confirm the abnormalities.

Subjects with confirmed ALT or AST levels >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN) should have liver chemistry tests (ALT, AST, AP, INR, and total bilirubin) retested at least twice weekly until ALT and AST levels become ≤1.2 × ULN, or 1.2 × baseline value if the baseline value was >ULN. In addition, the following evaluations should be performed:

1. Obtain a more detailed history of symptoms and prior and concurrent diseases.
2. Obtain further history for concomitant drug use (including nonprescription medications, herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
3. Obtain a history for travel and exposure to environmental chemical agents.
4. Serology for viral hepatitis (hepatitis A virus immunoglobulin M [IgM], hepatitis B surface antigen, hepatitis C virus antibody, cytomegalovirus IgM, and Epstein-Barr antibody panel).
5. Serology for autoimmune hepatitis (e.g., antinuclear antibody).

Additional liver evaluations, including gastroenterology/hepatology consultations, or hepatic computed tomography or magnetic resonance imaging (MRI), may be performed at the discretion of the investigator in consultation with the sponsor medical monitor. Repetition of the above evaluations should be considered if a subject's ALT and/or AST levels reach 5 × ULN.

8. STUDY PROCEDURES

A complete schedule of assessments is provided in Table 1. Descriptions of all required study procedures are provided in this section. In addition to protocol-mandated assessments, subjects should be followed per institutional guidelines, and unscheduled assessments should be performed when clinically indicated.

Missed evaluations should be rescheduled and performed as close to the originally scheduled date as possible except if rescheduling becomes, in the investigator's opinion, medically unnecessary or unsafe because it is too close in time to the next scheduled evaluation. In that case, the missed evaluation will be recorded as a protocol deviation and should be abandoned.

For the purposes of this protocol, there is no Day 0. All visit dates and windows are to be calculated using Day 1 as the date of CTX310 infusion.

8.1. Subject Screening, Enrollment, and Withdrawal

8.1.1. General Study Periods

8.1.1.1. Screening and Enrollment

Investigators will keep a log of all potential subjects reviewed and evaluated for study participation. Enrolled subjects are defined as subjects who consent to participate in the clinical study and whose eligibility is confirmed (meet the inclusion/exclusion criteria). Screen failures are defined as subjects who consent to participate in the clinical study but do not meet the eligibility criteria.

The screening period begins on the date that the subject signs the ICF and continues through confirmation of eligibility and enrollment into the study. Once informed consent has been obtained, the subjects will be screened to confirm study eligibility, as outlined in the schedule of assessments (Table 1). All screening assessments should be completed within 42 days after a subject signs the ICF. The medical monitor will review eligibility packets and verify information provided by the site to confirm agreement with the investigator that the subject is eligible for enrollment.

For the subject's convenience, if an assessment was performed before signing the ICF as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe.

Repetition of individual screening assessment(s) that did not meet eligibility requirements is not permitted with the following exceptions:

- If there is clear evidence of a laboratory error (e.g., hemolyzed sample) or equipment malfunction, collection of a repeat sample for the appropriate laboratory test or assessment may be permitted with the approval of the medical monitor.
- Individual laboratory results that, in the opinion of the investigator, are related to a temporary, reversible condition may be retested once after the condition resolves or within 7 days, whichever is earlier.

If repeat values of the individual assessment(s) are within the eligibility criteria and completed within the screening window, then the subject is eligible for the study.

Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

Specific procedures for enrollment will be provided to sites.

8.1.1.2. Infusion of CTX310

All subjects will receive a pretreatment regimen and study treatment, as described in Section 6.1.

8.1.1.3. Acute Safety Evaluation Period

The acute safety evaluation period is 30 days following infusion of CTX310 for each subject.

8.1.1.4. Follow-up and End of Study Definition

Following the 30-day acute safety evaluation period, subjects will be followed for an additional 11 months. Subjects will be considered to have completed the study after they complete the end of study (EOS) visit at Month 12.

The EOS is defined as the time at which the last subject completes the Month 12 visit, is considered lost to follow-up, withdraws consent, or dies.

8.1.1.5. Long-term Follow-up

To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310 and either discontinue prior to 12 months or complete this study will be asked to participate in a separate LTFU study for up to 15 years post-infusion to assess long-term safety, durability, and the occurrence of any clinical AE, including pancreatitis events and cardiovascular events.

8.1.2. Subject Identification

A unique subject number will be assigned when an individual subject signs the study ICF. Subjects will be identified by a subject number consisting of:

> Last 3 digits of protocol number (400), assigned 3-digit site number, sequential 3-digit subject number (e.g., 400-XXX-YYY)

Once a number is assigned to a subject, it cannot be reassigned to a different subject. Rescreened subjects will keep the same subject number assigned during the initial screening process.

8.1.3. Replacement of Subjects

Subjects must receive CTX310 to be evaluated for a DLT. If a subject discontinues the study at any time prior to CTX310 infusion, the subject will be deemed unevaluable for DLT and will be replaced. If a DLT-evaluable subject (i.e., a subject who has been administered CTX310) has

signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared.

Subjects who discontinue from the study at any other time post–CTX310 infusion for any other reason will not be replaced.

8.1.4. Subject Withdrawal or Discontinuation

Subjects may voluntarily withdraw from the study at any time. Withdrawal of full consent means that the subject does not wish to receive further protocol-required therapy or undergo any study procedures. The sponsor will be notified of all study withdrawals. Subject data and samples collected up to the date of withdrawal of consent will be retained and included in the analyses. Where permitted by local regulations, publicly available data (e.g., death records) may be included after withdrawal of consent.

The investigator will specify reason for discontinuation from the study as follows:

- Subject withdrawal of consent.
- Investigator decision (only for subjects who did not receive study drug).
- Loss to follow-up.
- Death.

For subjects who are lost to follow-up (defined as 3 documented attempts to contact the subject via all available contact information in a reasonable time period), the investigator should attempt to search publicly available records (where permitted and allowed by local law) to ascertain vital status. For the duration of the study, attempts should also be made to collect information from other sources related to hospitalizations.

Subjects who withdraw completely from this study will be asked to participate in the separate LTFU study if the LTFU has been approved at the investigative site. As CTX310 is not a continuously dosed investigational product, withdrawal from the study due to an AE is not applicable.

8.2. Study Assessments and Procedures

Refer to the schedule of assessments (Table 1) for the timing of the required procedures.

If needed, procedures may occur on separate days, but they must be done within the defined visit window.

8.2.1. Informed Consent

The investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, and possible risks and benefits of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated ICF must be obtained before conducting any study procedures (Section 13.4).

All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to rollover into a separate LTFU study for up to 15 years post-infusion (see Section 8.1.1.5 and Table 1).

Whenever important new information becomes available that may be relevant to the subject's consent, the written ICF and any other written information provided to subjects will be revised by the sponsor or designee and submitted to the Institutional Review Board (IRB)/Ethics Committee (EC) for review. The agreed upon, revised ICF will be provided to each subject in the study for signing and dating. The investigator will explain the changes to the previous version.

8.2.2. Demographics and Medical History

Demographic data, including date of birth, sex, race, and ethnicity, will be collected. Medical history, including a full history of the subject's disease and response to treatment from date of diagnosis, will be obtained. Cardiac and surgical history will also be obtained.

Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment and during screening will be collected.

For study entry, all subjects must fulfill all inclusion criteria described in Section 4.1, and have none of the exclusion criteria described in Section 4.2.

8.2.3. Physical Exam, Height, and Weight

Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, Day 1, Day 30, and EOS visits, and the results documented.

Symptom-directed abbreviated physical examination may be performed at all other study visits. Changes noted from the examination performed at screening will be recorded as AEs.

Weight will be obtained according to the schedule of assessments (Table 1).

Height, BMI, and waist/hip ratio will only be obtained at screening and EOS.

8.2.4. Vital Signs

Vital signs will be recorded at every study visit according to the schedule of assessments (Table 1), and will include a single measurement of blood pressure, heart rate, respiratory rate, oxygen saturation by pulse oximetry, and temperature. On Day 1 and during hospitalization, measurements will be performed at the time points specified in the schedule of assessments (Table 1). Blood pressure and pulse rate should be measured using the site's equipment. The following guidance is recommended during taking vital signs.

1. Subject should be seated in a chair with their back supported, feet flat on the floor, and arms bared and supported at heart level.
2. The appropriate cuff size must be used to ensure accurate measurement and used consistently throughout the study.

3. Readings should be done on the same arm at each visit, preferably the non-dominant arm.
4. Measurement should begin after at least 5 minutes of rest.
5. Assessment of pulse rate can be manual or automated. When done manually, the rate should be counted in the brachial/radial artery for at least 30 seconds.

Other procedures should not be performed during blood pressure or heart rate measurements.

Vital sign measurements may be performed by the subject`s health care provider and reported to the investigator.

8.2.5. Liver Imaging

Standard local procedures will be used for image acquisition and analysis. A liver FibroScan or MRE (depending on availability) will be performed at screening and results will be used to exclude patients with liver stiffness consistent with signs of fibrosis (Section 4.2, Exclusion Criterion 2c). A 3-hour fast is recommended prior to FibroScan. A liver MRI–PDFF (for assessment of fatty liver/steatosis) will be performed at screening and EOS visits. Baseline liver fat status and quantitative change in hepatic steatosis will be collected within the case report form (CRF), with clinically significant findings reported as medical history or AEs, as appropriate.

8.2.6. Echocardiogram

A transthoracic echocardiogram is required at screening for assessment of LVEF for all subjects. (see Schedule of Assessment, Table 1). Additional echocardiograms may be obtained at the investigator's discretion.

8.2.7. Electrocardiogram

Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen. QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion.

8.2.8. Laboratory Tests

Laboratory samples will be collected and analyzed according to the schedule of assessments (Table 1). Unless stated, local laboratories meeting country-specific requirements for clinical testing will be utilized to analyze all tests. Laboratory assessments are listed in Table 3 and Table 4.

Table 3: Local Laboratory Testing

Serum chemistry	ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN/urea, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein
Cardiac biomarker	Troponin I or T
Urinalysis	Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal), ACR[1]
Coagulation	PT (INR), PTT/aPTT, fibrinogen
Serum or urine pregnancy test	hCG
CBC with differential	Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count, absolute lymphocyte count Note: The white blood cell count differential may be reported as absolute counts or as percentages, with the exception that absolute neutrophil and lymphocyte counts are required for eligibility assessment.
Thyroid function	T3, T4, TSH
Viral serology	HIV-1, HIV-2, HCV antibody or NAT, HBV surface antigen, HBV surface antibody, HBV core antibody or NAT

ACR: albumin to creatinine ratio; aPTT: activated partial thromboplastin time; ALT: alanine aminotransferase; AST: aspartate aminotransferase; BUN: blood urea nitrogen; CBC: complete blood count;eGFR: estimated glomerular filtration rate; HbA1c: glycosylated hemoglobin; HBV: hepatitis B virus; hCG: human chorionic gonadotropin; HCV: hepatitis C virus; HIV: human immunodeficiency virus; INR: international normalized ratio; MDRD: Modification of Diet in Renal Disease; NAT: nucleic acid testing; PT: prothrombin time; PTT: partial thromboplastin time; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; T3: triiodothyronine; T4: thyroxine; TSH: thyroid-stimulating hormone.
[1] Albuminuria (dipstick) or ACR to be performed at screening as specified in the schedule of assessments (Table 1).

Table 4: Central Laboratory Testing

Genetic testing[1]	*LDLR*, *APOB*, *PCSK9, LPL*, *APOC2*, *APOA5*, *LMF-1*, *GPIHBP1*
Lipid panel	Total cholesterol, TG, HDL-C, non–HDL-C, LDL-C, VLDL-C, Lp(a), ApoB, ApoC-III [2, 3]

APOA5: apolipoprotein A5; ApoB or *APOB*: apolipoprotein B; *APOC2*: apolipoprotein C2; ApoC-III: apolipoprotein C-III; *GP1HBP1*: glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; *LDLR*: low-density lipoprotein receptor; *LMF-1*: lipase maturation factor 1; Lp(a): lipoprotein(a); LPL: lipoprotein lipase; *PCSK9*: proprotein convertase subtilisin/kexin type 9; TG: triglyceride(s); VLDL-C: very low-density lipoprotein cholesterol.
[1] Collection of a sample for genetic testing should not be repeated during rescreening, if applicable.
[2] Subjects on apheresis should have lipid levels sampled within 5 days prior to the procedure (a pre-apheresis sample on the day of apheresis is also adequate).
[3] All lipid panel samples testing to be performed after a minimum of an 8-hour fast.

8.2.9. Pregnancy Testing

All females must have pregnancy tests performed according to the schedule of assessments (Table 1). Serum pregnancy testing will be performed at screening. Urine or serum pregnancy test can be performed as per local standards at the EOS/M12 visit (Table 1, Table 3).

8.3. Immunogenicity

CTX310 is composed of mRNA encoding SpCas9 and [redacted] sgRNA targeting the gene of interest, encapsulated in an LNP. Blood samples will be collected as described in the schedule of assessments (Table 1) and stored for potential future immunogenicity assessments (anti-drug antibody [ADA] to LNP and Cas9), if required.

8.4. Pharmacokinetics and Pharmacodynamics Assessments

8.4.1. CTX310 Pharmacokinetic Analysis

PK analysis of [redacted](LNP), [redacted] (LNP), and Cas9 protein levels will be performed on blood samples collected per the schedule of assessments (Table 1). On Day 1, samples will be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at the scheduled time points after the completion of CTX310 infusion, as described in the schedule of assessments (Table 1). For all other time points specified in the schedule of assessments (Table 1), including D2, D3, D4, a single sample will be collected as described.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.2. ANGPTL3

Plasma samples will be obtained to follow the ANGPTL3 concentration, as described in the schedule of assessments (Table 1).

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3. Exploratory Biomarker Research

Exploratory biomarker research may be conducted to identify genomic, metabolic, and/or proteomic biomarkers that may be indicative or predictive of clinical response, resistance, safety, PD activity, and/or the mechanism of action of treatment. In addition, samples collected for protocol-specific endpoints will be used for exploratory research, pending availability of excess sample.

The sponsor may request discontinuation of sample collection. Continue sample collection for all listed time points until otherwise instructed by sponsor.

8.4.3.1. Whole Blood

Whole blood samples will be obtained and stored at screening.

8.4.3.2. Plasma

Plasma samples for storage will be obtained at screening.

8.4.3.3. Serum

Serum samples will be obtained to follow exploratory biomarkers (e.g., cytokines), as described in the schedule of assessments (Table 1).

9. SAFETY, ADVERSE EVENTS, AND STUDY OVERSIGHT

The investigator will monitor each subject for clinical and laboratory evidence of AEs on a routine basis throughout the study and assess and record details as described in Section 9.1. AEs in response to a query, observed by site personnel, or reported spontaneously by the subject will be recorded. The investigator is responsible for ensuring that any AEs observed by the investigator or reported by the subject are recorded in the subject's medical record in the sponsor's electronic data capture system. AEs will be followed through to event resolution or stability or death.

9.1. Adverse Events

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not considered related to the medicinal (investigational) product [Guidelines for Good Clinical Practice (GCP) E6(R2)]. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

The investigator will assess and record information pertaining to the AE, which includes but is not limited to the following: date of onset, event diagnosis (when known) and/or signs and symptoms, duration, severity, seriousness, relationship to the study therapy or procedure, action(s) taken, and outcome.

Additional criteria defining an AE are described below.

The following **are** considered AEs:

- Aggravation of a pre-existing disease or permanent disorder (any clinically significant worsening in the nature, severity, frequency, or duration of a pre-existing condition).
- Events resulting from protocol-mandated procedures (e.g., complications from invasive procedures).

The following **are not** considered AEs:

- Elective or preplanned medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. These should be recorded in the relevant eCRF.

  Note: An untoward medical event occurring during the prescheduled elective procedure or routinely scheduled treatment should be recorded as an AE or SAE.
- Pre-existing diseases or conditions that do not worsen during or after administration of the investigational medicinal product.
- Hospitalization planned for study treatment infusion or observation.

The investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual study subject represents a clinically significant change from the subject's baseline value. Only abnormal laboratory results considered by the investigator to be clinically significant should be reported as AEs (e.g., an abnormal laboratory finding associated with clinical symptoms, of prolonged duration, or that requires additional monitoring and/or medical intervention). Whenever possible, these should be reported as a clinical diagnosis rather than the abnormal parameter itself (i.e., neutropenia vs neutrophil count decreased). Abnormal laboratory results without clinical significance should not be recorded as AEs.

AEs can occur before, during, or after treatment, and can be either treatment-emergent (AEs that start or worsen on or after CTX310 infusion) or non–treatment-emergent. A non–treatment-emergent AE is any new sign or symptom, disease, or other untoward medical event that occurs after written informed consent has been obtained and before the subject has received CTX310.

9.2. Serious Adverse Event

An AE of any untoward medical consequence must be classified as an SAE if it meets any of the following criteria:

- Results in death.
- Is life-threatening (i.e., an AE that, in the opinion of the investigator, places the subject at immediate risk of death).
- Requires inpatient hospitalization or prolongs an existing hospitalization (hospitalizations for scheduled medical or surgical procedures or to conduct scheduled treatments do not meet these criteria).
- Results in persistent or significant disability or incapacity.
- Results in a congenital anomaly or birth defect in a newborn.
- Other important/significant medical events. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Hospitalization for study treatment infusions or planned hospitalizations following CTX310 infusion are not considered SAEs. Furthermore, hospitalizations for observation or prolongation of hospitalization for observation alone should not be reported as an SAE unless they are associated with a medically significant event that meets other SAE criteria, as assessed by the investigator.

9.3. Adverse Events of Special Interest

An AESI, whether serious or nonserious, is one of scientific and medical concern specific to the sponsor's product for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate.

The following events and/or laboratory findings will be designated as AESIs based on the predicted pharmacology, nonclinical safety profile, possible off-target effects, and/or adverse reactions seen in studies of other ANGPTL3 inhibitors (see Investigator's Brochure for details):

- IRRs.
- Abnormal coagulation findings, defined as clinically relevant abnormal bleeding, thrombotic, or hemorrhagic events.
- Increase in ALT or AST: ≥3 × ULN or ≥2 × the baseline value (if baseline ALT ≥ULN).
- Allergic reactions/events or localized reactions (collected for 30 days post-infusion).
- New malignancy.

Additional information on the required AESI reporting collection period is detailed in Table 6.

9.4. Adverse Event Severity

AESIs and DLTs will be graded using CTCAE version 5.0. If CTCAE v5.0 grade or protocol-specified criteria are not applicable, AE toxicity should be graded according to Table 5.

Table 5: Adverse Event Severity

Severity Grade	Description
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental ADL. [1]
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL. [2]
4	Life-threatening consequences: urgent intervention indicated.
5	Death related to AE.

ADL: activities of daily living; AE: adverse event.
[1] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[2] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

9.5. Adverse Event Causality

The investigator must assess the relationship between each AE and CTX310 and any protocol-mandated study procedure (all assessed individually). The assessment of relationship will be made based on the following definitions:

- **Related**: There is a clear causal relationship between the study treatment or procedure and the AE.
- **Not related**: There is no evidence to suggest a causal relationship between the study treatment or procedure and the AE.

Investigators should consider the temporal association between the timing of the event and administration of the treatment or procedure, a plausible biological mechanism, and other potential causes of the event (e.g., concomitant therapy, underlying disease) when making their assessment of causality.

If an AE is assessed to be not related to any study intervention, an alternative etiology must be provided in the CRF.

9.6. Outcome

The outcome of an AE will be classified and reported as follows:

- Fatal.
- Not recovered/not resolved.
- Recovered/resolved.
- Recovered/resolved with sequelae.
- Recovering/resolving.
- Unknown.

9.7. Adverse Event Collection Period

The safety-related information of all subjects in this study will be recorded from the time of ICF signing until EOS/M12; however, there are different reporting requirements for the different time periods in the study. Table 6 describes the AEs that should be reported at each time period of the study.

Table 6: Adverse Event Collection by Study Time Period

Time Period	AE Reporting Requirements
Informed consent to 30 days after CTX310 infusion	All AEs
30 days after CTX310 infusion through Month 12 visit	• Nonserious AEs related to study procedure[1] or CTX310 • SAEs • AESIs

AE: adverse event; AESI: adverse event of special interest; SAE: serious adverse event.
[1] AEs related to study procedures include events related to, e.g., laboratory procedures, imaging, or prophylaxis regimen, and may occur at any time starting at screening and continuing through the duration of the study.

If a subject does not receive CTX310 therapy, the AE reporting period ends.

9.8. Adverse Event Reporting

All AEs will be recorded in the appropriate section of the eCRF. Subjects withdrawn from the study because of AEs will be followed by the investigator until the outcome is determined. When appropriate, additional written reports and documentation will be provided.

AE reporting should occur as per the study period designated in Table 6. If a reportable SAE or AESI occurs, the SAE/AESI form provided to investigators should be completed and submitted to the sponsor or its designee immediately (i.e., no more than 24 hours after the investigator becomes aware of the event) by scanning and emailing the paper report to:

globalpv@crisprtx.com (for notifications or questions)

In particular, if the SAE is fatal or life-threatening, the report form must be submitted immediately, irrespective of the extent of available AE information. The timeframe also applies to additional, new information (follow-up) on previously reported SAE/AESI reports.

In the rare event that the investigator does not become aware of the occurrence of a reportable SAE/AESI immediately (e.g., a study subject initially seeks treatment elsewhere), the investigator is to report the event within 24 hours of learning of the event and document the date and time of awareness of the event.

In addition, an investigator may be requested to obtain specific additional follow-up information in an expedited fashion (e.g., autopsy finding). The information collected for SAEs/AESIs is more detailed than that captured on the AE CRF. In general, this will include a description of the event in sufficient detail, as well as concomitant medications and any relevant medical details to allow for a complete medical assessment of the case and causality assessment by the investigator and the sponsor. Information on the other possible causes of the event, such as concomitant medications and illnesses must be provided.

The investigator must complete, sign, and date the SAE/AESI form, verify the accuracy of the information recorded on the SAE/AESI form with the corresponding source documents, and send

a copy by email or fax to the sponsor or designee. Subsequently, all SAEs/AESIs will be reported to the health authorities per local reporting guidelines.

It is the principal investigator's responsibility to notify the IRB or EC of all SAEs that occur at his or her site as per local policies. Investigators will also be notified of all unexpected, serious, drug-related events that occur during the clinical study. Each site is responsible for notifying its IRB or EC of these additional SAEs.

9.9. Medication Errors and Overdose

An overdose or medication error along with any associated AE, regardless of seriousness, should be reported to the sponsor on the provided SAE/AESI form within 24 hours of awareness of the medication error/overdose or associated AE.

9.10. Pregnancy

Certain information, although not considered an AE or SAE, must be recorded, reported, and followed as indicated for an SAE (see Section 9.8), including pregnancies.

Pregnancies (both those of female subjects and female partners of male subjects) must be reported to the sponsor or designee within 24 hours of the investigator's knowledge using the Investigational Product Pregnancy Report. All pregnancies will be followed through outcome and the infant will be followed at birth and at 1 year, provided informed consent is obtained. Pregnancy outcome must be reported to the sponsor or designee using the pregnancy outcome section of Investigational Product Pregnancy Report.

Pregnancies themselves are not considered AEs or SAEs. However, any AEs or SAEs occurring during pregnancy are to be reported following AE and SAE reporting guidelines. Any congenital anomalies and birth defects in the infant should be reported as an SAE. Additionally, any CTX310-related malignancy or death in the infant during the 12-month follow-up period should also be reported as an SAE.

9.11. Reporting Deaths

Regardless of relationship to the investigational product, all deaths on study should be recorded in the relevant eCRF. Additionally, all AEs with an outcome of death, regardless of relationship to investigational product, should be reported to the sponsor as SAEs, to Pharmacovigilance on the provided SAE/AESI form.

9.12. Study Oversight

9.12.1. Safety Review Committee

During dose escalation, an SRC consisting of investigators and sponsor representatives will review all available safety data and make decisions regarding dose escalation or de-escalation. During dose escalation, the SRC may also propose revisions to the DLT definitions and dosing schema and will continue to meet regularly to discuss toxicity management algorithms and review individual subject cases. Following discussion with the SRC, the sponsor may consult

with the independent DSMB regarding emergent safety data and discuss potential revisions to DLT criteria or alternate dosing schema.

During follow-up, the SRC may continue to meet on an ad hoc basis to discuss 1 or more of the following: (1) single-subject case studies, (2) aggregate safety and/or biomarker data, (3) toxicity management algorithms, and (4) review and discuss the possibility of expanding the study to include other study populations.

The SRC may be consulted on other aspects of the study conduct, as applicable.

Any revisions to the DLT definitions or criteria, dosing schema can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented.

9.12.2. Independent Data Safety Monitoring Board

An independent DSMB comprised of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise to monitor the study will be established during dose escalation. Throughout the study the DSMB will review safety data from Phase 1a dose escalation and Phase 1b disease-specific cohort expansion. The DSMB will review safety data pertaining to stopping rules provided by the sponsor, as detailed in the DSMB charter. The DSMB may recommend that the sponsor amend the protocol, stop enrollment, or discontinue the study at any time if concerns about safety of the subjects are encountered. The roles and responsibilities of the DSMB will be further described in the DSMB charter.

Any DSMB recommended revisions to the protocol can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented.

10. STOPPING RULES AND STUDY TERMINATION

10.1. Stopping Rules for the Study

Administration of the investigational product will be paused temporarily for safety reasons, pending review of the data by the sponsor, SRC, and DSMB upon the occurrence of laboratory results meeting any of the criteria in Section 10.1.1. Laboratory values that are not confirmed due failure to retest or missing laboratory values will be presumed confirmed.

Subjects who have already received a dose of study drug will continue with follow-up as outlined in the protocol. No further dosing of subjects will occur until an assessment of the data is completed by the sponsor, the SRC, and the DSMB. Notification of the suspension of accrual will be provided to the health authorities and ECs. If deemed appropriate based on the sponsor, the SRC, and the DSMB review, a rationale with data supporting continued dosing without a change to the protocol will be submitted to the health authorities and ECs for review and approval. If a modification is required, a protocol amendment will be submitted. No changes to the protocol can be implemented until the application is approved by the regulatory authority.

If any stopping rule is met, the study can only be restarted after an amendment application that presents the data and the rationale to restart is approved by the regulatory authority.

10.1.1. Stopping Rules

Treatment of the next subject will be paused as described in Section 10.1 if any of the following criteria are met.

1. ≥2 subjects at a DL with ALT or AST >8 × ULN, which is confirmed and persists for >14 days.
2. ≥2 subjects at a DL with ALT or AST >5 × ULN, which is confirmed and persists for >30 days.
3. ≥2 subjects at a DL with ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5 × ULN persisting for >14 days.
4. Thrombosis, hemorrhage, and/or laboratory parameters consistent with disseminated intravascular coagulation or bleeding that are likely related to study drug.
5. Fatal or life-threatening AE that is due to investigational product.
6. Any other AE that poses an immediate hazard to study subjects, in the opinion of the sponsor in consultation with the investigators and SRC.

10.2. Stopping Rules for Individual Subjects

Stopping rules for individual subjects are as follows:

- Any medical condition that, in the opinion of the investigator or sponsor, would put the subject at risk during continuing study-related treatments or follow-up.

- If a subject is found to have not met all the eligibility criteria or has a major protocol deviation before the start of CTX310 infusion and in the opinion of the investigator.
- Would put the subject at risk for continuing study-related procedures or follow-up.

10.3. Study Termination

This study may be discontinued at any time due to safety concerns, failure to meet expected enrollment goals, administrative reasons, or at the discretion of the sponsor. In the event of study termination, the sponsor will immediately inform all appropriate parties, including principal investigators, ECs, IRBs, and competent authorities. In the event this study is terminated early, subjects who have received CTX310 will be asked to participate in a separate LTFU study for up to 15 years post-infusion.

11. STATISTICAL ANALYSES

11.1. Study Objectives and Hypotheses

The primary objective is to evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of LDL-C and to determine the RP2D.

The secondary objectives are to further characterize the safety and tolerability, and to assess the preliminary efficacy, PK, and PD of CTX310. No formal hypothesis testing will be performed.

11.2. Study Endpoints

11.2.1. Primary Endpoints

- Incidence of DLTs

11.2.2. Secondary Endpoints

11.2.2.1. Secondary Efficacy Endpoints

- Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline.

11.2.2.2. Secondary Safety Endpoints

- Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs

11.2.2.3. Secondary Pharmacokinetics/Pharmacodynamics Endpoints

- Assess the plasma levels of LNP ([redacted] and [redacted]).
- Assess the plasma level of Cas9 protein.
- Percentage change in ANGPTL3 concentration over time compared to baseline.

11.2.3. Exploratory Endpoints

- Percentage change in free fatty acid (FFA) levels over time compared to baseline.
- Change in fatty liver disease.
- Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required).

For Phase 1b subjects only

- Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG.

11.3. Analysis Sets

The following analysis sets will be evaluated and used for presentation of the data.

- The enrolled set includes all subjects who sign the informed consent and meet the inclusion/exclusion criteria.
- The safety analysis set is a subset of the enrolled set that includes subjects who receive the CTX310 infusion. Analyses of the safety assessments will be based on the safety analysis set. Subjects in the safety analysis set will be classified by received CTX310 DL.
- The DLT evaluable set includes all subjects during dose escalation who receive CTX310 infusion and complete the DLT evaluation period or discontinue early after experiencing a DLT. The DLT period will begin with CTX310 infusion and last for 30 days, or beyond for improvement or resolution of the signs or symptoms of a potential DLT. The DLT evaluable set will be used for dose escalation decisions.
- The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL.

11.4. Sample Size

The sample size of the study will be approximately 69 subjects. This will include approximately 21 subjects in Phase 1a dose escalation and approximately 48 subjects in the Phase 1b disease-specific cohort expansion parts of this study. In Phase 1b, there will be up to 12 subjects per disease-specific cohort.

No formal sample size calculation was performed for this study. The total sample size is estimated based on the dose escalation/expansion design and considered sufficient for the preliminary evaluation of the safety and tolerability of CTX310.

11.5. Interim Analysis

No formal efficacy interim analysis is planned.

The DSMB will review safety and efficacy data as needed during the study to monitor stopping rules and to provide recommendations on enrollment or protocol amendment.

11.6. Planned Method of Analyses

The primary analysis will occur after all subjects have completed 26 weeks of follow-up after CTX310 infusion or discontinued earlier. A final analysis will occur when all subjects complete or withdraw from the study. Tabulations will be produced for appropriate disposition, demographic, baseline, efficacy, and safety parameters. By-subject listings will be provided for all data, unless otherwise specified.

11.6.1. Efficacy Analysis

The FAS will be used as the analysis set for efficacy. The efficacy endpoints of percentage change in lipid concentrations, including TG, ApoB, non–HDL-C, LDL-C, and HDL-C, over time compared to baseline will be summarized using descriptive statistics for each DL. Categorical summaries at selected time points, including 26 and 52 weeks, based on appropriate cutoff may be provided.

11.6.2. Safety Analysis

Safety analysis will be conducted on the safety analysis set. Summaries of DLTs and other AEs, AESIs, clinical laboratory data, and other applicable safety measures (e.g., ECG) will be provided for each DL of CTX310 and overall.

Summaries of AEs will focus on TEAEs, defined as AEs that start or worsen on or after CTX310 infusion. AEs will be graded according to CTCAE v5.0. The incidence of TEAEs will be summarized by system organ class and preferred term, grade, and relation to CTX310. Key subsets of TEAEs, including DLTs, AESIs, grade ≥3 AEs, related AEs, and SAEs, will be summarized separately.

Summaries of clinical laboratory data will include descriptive statistics of absolute value and/or change from baseline at scheduled visits for selected laboratory parameters. The incidence of clinically significant laboratory abnormalities and clinically significant abnormal vital signs will be summarized.

The incidence of other clinically significant safety measure abnormalities (e.g., ECG) will also be summarized, if applicable.

11.6.3. Pharmacokinetic and Pharmacodynamic Analyses

Plasma levels of LNPs ([redacted] and [redacted]) and Cas9 protein over time will be summarized using descriptive statistics. Exploratory analysis based on an applicable PK model may be performed.

Percentage change in ANGPTL3 concentration over time compared to baseline will be summarized using descriptive statistics.

11.6.4. Biomarker Analyses

Additional exploratory biomarkers, including FFA levels, fatty liver disease marker(s), and immunogenicity marker(s), if data are available, will be summarized using descriptive statistics.

11.6.5. Exploratory Analysis of Pancreatitis Event

The annualized event rate of pancreatitis after CTX310 will be estimated using Poisson model and compared to baseline to assess the effect of CTX310 on pancreatitis event.

11.6.6. Patient-reported Outcome Analyses

Not applicable.

12. DATA MANAGEMENT

12.1. Data Recording and eCRF Processing

The investigator is required to maintain adequate and accurate medical records designed to record all observations and data pertinent to the study for each subject. Study data for each consented subject will be entered into a CRF by site personnel using a secure, validated (Part 11 of Title 21 of the Code of Federal Regulations–compliant), web-based electronic data capture application. Instances of missing, discrepant, or uninterpretable data will be queried by the sponsor or designee for resolution. Any changes to study data will be made to the eCRF and documented in an audit trail maintained within the clinical database. CRFs must be reviewed and electronically signed and dated by the investigator.

An audit may be performed at any time during or after completion of the clinical study by sponsor personnel or their designee. All study-related documentation must be made available to the designated auditor.

13. ADMINISTRATIVE

13.1. Institutional Review Board/Ethics Committee

This protocol and the proposed ICF must be reviewed and approved by the appropriate IRB/EC prior to the start of the study. During the study, the investigator shall make timely and accurate reports to the IRB/EC on the progress of the study at intervals not exceeding 1 year, as well as satisfying any other local IRB/EC regulations regarding reporting. Copies of all reports to and correspondence with and from the IRB/EC must be provided to the sponsor or its designee.

Any significant changes or revisions in the study protocol or any changes that may alter subject risk must be approved in writing by the IRB/EC prior to implementation. A protocol change intended to eliminate an apparent imminent hazard may be implemented immediately provided that the sponsor is promptly notified, and an amendment is subsequently provided by the sponsor and approved by the IRB/EC.

It is the investigator's obligation to maintain an IRB/EC correspondence file, and to make this available for review by sponsor representatives or their designee as part of the study monitoring process.

13.2. Study Conduct

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with International Conference on Harmonisation (ICH) Guidelines for GCP and applicable regulatory requirements.

13.3. Subject Privacy

To maintain subject confidentiality and to comply with applicable data protection and privacy laws and regulations, all data provided to the sponsor or designee, study reports, and communications relating to the study will identify subjects by assigned subject numbers, and access to subject names linked to such numbers shall be limited to the site and the study investigator and shall not be disclosed to the sponsor or designee. As required by applicable laws and regulations in the countries in which the study is being conducted, the investigator will allow the sponsor and/or its representatives access to all pertinent medical records to allow for the verification of data gathered and the review of the data collection process. The regulatory authorities in other jurisdictions, including the IRB/EC, may also request access to all study records, including source documentation, for inspection.

13.4. Written Informed Consent

The investigator will be responsible for obtaining written informed consent from potential subjects prior to any study-specific screening and entry into the study. The source documents for each subject shall document that the informed consent was obtained prior to participation in the study.

The investigator at each center will ensure that the subject is given full and adequate oral and written information about the nature, purpose, and possible risk and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject

should be given the opportunity to ask questions and allowed time to consider the information provided. The investigator must maintain the original, signed ICF. A copy of the signed ICF must be given to the subject. Whenever important new information becomes available that may be relevant to the subject's consent, the written ICF and any other written information provided to subjects will be revised by the sponsor or designee and be submitted to the IRB/EC for review and favorable opinion. The agreed upon, revised information will be provided to each subject in the study for signing and dating. The investigator will explain the changes to the previous version.

13.5. Delegation of Investigator Responsibilities

The investigator will ensure that all persons involved in the conduct of the study are informed about the protocol, protocol amendments, study procedures, and study-related duties.

13.6. Study Files

Documentation concerning investigators' credentials and experience, and IRB approval of protocol and ICF, and other documentation are required prior to shipment of the investigational product to the study site. Copies of these documents as well as supplemental information, such as the Investigator's Brochure, will be kept onsite in an investigator study file binder. This file also will contain investigational product accountability (receipt/dispensing) records, sponsor/investigator correspondence, IRB correspondence, changes to the protocol, information regarding monitoring activities, subject exclusion records, and biological sample records.

13.7. Retention of Study Documents

All study documents, including records of investigational product receipt and disposition, copies of eCRFs, as well as supporting documentation and administrative records, must be retained by the investigator for a minimum of 2 years following notification that the appropriate regulatory authority has approved the product for the indication under study, notification that the entire clinical investigation will not be used in support of a marketing application, or notification that the marketing application was not approved. No study documents will be destroyed or moved to a new location without prior written approval from the sponsor. If the investigator relocates, retires, withdraws from the clinical study for any reason, or dies, all records required to be maintained for the study should be transferred to an agreed upon designee, such as the study monitor, another investigator, or the institution where the study was conducted. The sponsor should be notified in writing at least 30 days prior to the disposal of any study records related to this protocol.

13.8. Protocol Compliance

No modifications to the protocol will be made without the approval of both the investigator and the sponsor. Changes that significantly affect the safety of the subjects, the scope of the investigation, or the scientific quality of the study (e.g., efficacy assessments) will require IRB/EC notification before implementation, except where the modification is necessary to eliminate an apparent immediate hazard to human subjects. The sponsor will submit all protocol modifications to the required regulatory authorities.

Emergency departures from the protocol that eliminate an apparent immediate hazard to a particular subject and that are deemed crucial for the safety and well-being of that subject may be instituted for that subject only. The investigator or other attending physician also will contact the sponsor as soon as possible in the case of such a departure. These departures do not require preapproval by the IRB; however, the IRB and sponsor must be notified in writing as soon as possible after the departure has been made. In addition, the investigator will document the reasons for protocol deviation and the ensuing events.

13.9. Monitoring Functions and Responsibility

Before an investigational site can enter a subject into the study, a sponsor representative will evaluate the investigational study site to:

- Determine the adequacy of the facilities.
- Discuss with the investigator(s) and other personnel their responsibilities regarding protocol adherence, and the responsibilities of the sponsor or its representatives. This will be documented in a Clinical Study Agreement between the sponsor and the investigator.

During the study, a monitor from the sponsor or representative will have regular contacts with the investigational site to:

- Provide information and support to the investigator(s).
- Confirm that facilities remain acceptable.
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, and that investigational product accountability checks are being performed.
- Perform source data verification. This includes a comparison of the data in the CRFs with the subject's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each subject (e.g., clinic charts).
- Record and report any protocol deviations not previously sent to the sponsor.
- Confirm AEs and SAEs have been properly documented on CRFs, that any SAEs and AESIs have been forwarded to the sponsor, and SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the investigator(s) or other staff needs information or advice.

13.10. Quality Control and Quality Assurance

Authorized representatives of the sponsor, a regulatory authority, an EC, or an IRB may visit the site to perform audits or inspections, including source data verification. The purpose of a sponsor audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded,

analyzed, and accurately reported per the protocol, ICH/GCP guidelines, and any applicable regulatory requirements. The investigator should contact the sponsor immediately if contacted by a regulatory agency about an inspection.

13.11. Disclosure of Data

All information obtained during the conduct of this study will be regarded as confidential. Disclosures (i.e., any release of information to any third party not noted herein) of any information not previously known to be public and/or results of the investigation for publication or by capsules or poster presentation shall not be made earlier than 30 days after submission of the proposed material to the sponsor for inspection, unless the sponsor consents to earlier disclosure. The investigator will take appropriate cognizance of the sponsor's suggestions before disclosure for publication or presentation consistent with protection of the sponsor's right to its confidential data.

13.12. Confidentiality and Publication

All scientific, commercial, and technical information disclosed by the sponsor in this protocol or elsewhere will be considered the confidential and proprietary property of the sponsor. The investigator will hold such information in confidence and shall not disclose the information to any third party except to such of the investigator's employees and staff as have been made aware that the information is confidential and who are bound to treat it as such and to whom disclosure is necessary to evaluate that information. The investigator will not use such information for any purpose other than determining mutual interest in performing the study and, if the parties decide to proceed with the study, for the purpose of conducting the study.

The investigator understands that the information developed from this clinical study will be used by the sponsor in connection with the development of the investigational product and other drugs and diagnostics and therefore may be disclosed as required to other clinical investigators, business partners and associates, the FDA, and other government agencies. The investigator also understands that to allow for the use of the information derived from the clinical study, the investigator has the obligation to provide the sponsor with complete test results and all data developed in the study.

Authorship of publications will be determined based on the Recommendations for Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, which states that authorship should be based on the following 4 criteria:

1. Substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data;
2. Drafting of the article or revising it critically for important intellectual content;
3. Final approval of the version to be published; and
4. Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

An individual must meet all criteria to be an author on any publication containing data from this study.

No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between the sponsor and the investigator and/or the investigator's institution.

13.13. Clinical Study Report

After completion of the study, a clinical study report, written by the sponsor or designee in accordance with the ICH E3 Guideline, will be submitted in accordance with local regulations.

14. APPENDIX: DEFINITIONS AND SUPPORTING OPERATIONAL DETAILS

14.1. Lean Body Weight Calculation

The eLBW will be estimated using the equation developed and validated by Janmahasatian et al, (Janmahasatian et al., 2005).

Females: LBW[kg] = (9270 × Total_body_weight[kg]) / (8780 + (244 × BMI[kg/m^2]))

Males: LBW[kg] = (9270 × Total_body_weight[kg]) / (6680 + (216 × BMI[kg/m^2]))

Where BMI[kg/m^2] = Total_body_weight[kg] / Height[m]^2

14.2. Contraception Requirements

Females of childbearing potential are excluded from the study.

Female Subjects of Nonchildbearing Potential

Female subjects of nonchildbearing potential will not be required to use contraception. To be considered of nonchildbearing potential, female subjects must meet at least 1 of the following criteria:

- Postmenopausal: At least 12 consecutive months of amenorrhea in women with a uterus without an alternative medical cause; **or**
- Surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy at least 1 month prior to screening).

Note: All other female subjects (including subjects with tubal ligations and subjects who do not have a documented hysterectomy) will be considered of childbearing potential.

Acceptable methods of contraception for male subjects and their partners are listed below. If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.

Male Subjects

Acceptable contraceptive methods must be used from study informed consent through 12 months after CTX310 infusion and include the following:

- Nonsterile male subjects who are or may become sexually active with female partners of childbearing potential must agree to use an acceptable, effective method of contraception from study informed consent through 12 months after CTX310 infusion.
- If the male is infertile (e.g., bilateral orchiectomy). Infertility may be documented through examination of a semen specimen or by demonstration of the absence of the vas deferens by ultrasound.

- True abstinence for the subject, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, postovulation methods) and withdrawal are not acceptable methods of contraception.
- Condom with spermicide (either as a single product if commercially available and/or allowed according to local regulations; otherwise, condom and spermicide as separate products). Local regulations may require use of an additional acceptable method of contraception. Note: Male condoms without spermicide may be used with another acceptable method of female contraception listed below (see acceptable contraceptive methods for female partners of male subjects).
- Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of enrollment, and 1 barrier method of contraception.

Acceptable Contraceptive Methods for Female Partners of Male Subjects:

- Bilateral tubal ligation performed at least 6 months previously.
- Continuous use of an intrauterine device for at least 90 days before consent.
- Hormonal contraceptives, if successfully used for at least 60 days before consent.

Additional Notes:

- Female condom cannot be used with male condom (as a double method of contraception) due to risk of tearing.
- The use of birth control methods does not apply if the female partner has had a bilateral oophorectomy, hysterectomy, or is postmenopausal.
- Male subjects who are not sexually active at the time of screening must agree to follow the contraceptive requirements of this study if they become sexually active with a partner of the opposite sex.
- If applicable, additional contraception requirements may need to be followed according to local regulations and/or requirements.
- Male subjects must not donate sperm throughout the study, and for 12 months following CTX310 infusion.
- Unique situations that may not fall within the above specifications may be discussed with the sponsor medical monitor or designee on an individual basis.

14.3. Country/Region-Specific Differences

Not applicable for this version of the protocol.

14.4. Protocol Amendments

The summary of key changes for the current amendment is located above the Table of Contents. The protocol is Version 6.1, Amendment 5 for the UK and replaces V5.1, Amendment 4 for the UK (17 January, 2025).

14.4.1. Summary of Changes to Current UK Specific Protocol Version 6.1

Change			Rationale	Affected Section(s)
Changes specific to Section 1.1 Protocol Synopsis (identical changes in the synopsis and the body of the protocol, Sections 2 through 14, are captured in chronological order by protocol body section level heading)				
Title Page - Administrative Update Original:			Administrative update	Title Page
Sponsor Emergency Contact	~~Sandeep Soni, MD Executive Director, Clinical Development CRISPR Therapeutics 455 Mission Bay Blvd South 3rd Floor San Francisco, CA 94158~~ USA	+1 ~~650-448-8044~~		
Modified to:				
Sponsor Emergency Contact	*Jason Duran, MD, PhD Head of Cardiovascular Clinical Development CRISPR Therapeutics 105 W First St Boston, MA 02127 USA*	*+1 617-315-4600*		
Section 1.1 Study Synopsis				
Section 1.1 Protocol Synopsis, Section 5.2 Number of Study Subjects, and Section 11.4 Sample Size – updated to accommodate addition of Phase 1b disease-specific cohort expansion. Original: Approximately ~~24~~ eligible subjects will be enrolled in the study. Modified to: Approximately *69* eligible subjects will be enrolled in the study. *Phase 1a Dose Escalation: Approximately 21 subjects* *Phase 1b Cohort Expansion: Approximately 48 (up to 12 subjects per disease-specific cohort).*			Updated sample size required to accommodate addition of Phase 1b disease-specific cohort expansion.	Section 1.1 Protocol Synopsis, Section 5.2 Number of Study Subjects, and Section 11.4 Sample Size
Study Population – addition of study design figure and text Addition of Figure S1 Study Design Modified to: "*The Phase 1a dose escalation will include eligible subjects, regardless of underlying dyslipidemia subtype.* The majority of subjects enrolled *in Phase 1a* are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia *and HTG*. Subjects with *elevated lipids of undetermined etiology* will *also be eligible for enrollment based on the overall known benefit of* lipid-lowering *treatments in reducing cardiovascular disease (CVD) risk.* *The Phase 1b cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD, that encompass HTG and/or hypercholesterolemia in the following disease-specific cohorts:*			Added text to describe Phase 1b	Section 1.1 Study Synopsis and Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
• *Severe HTG with history of ASCVD* • *HoFH* • *HeFH* • *Mixed hyperlipidemias with history of ASCVD* *Populations with ASCVD are intended to address secondary prevention with history of prior myocardial infarction (MI) or stroke or known subclinical atherosclerosis on computed tomography (CT) calcium score (>300), CT coronary arteries (obstructive coronary artery disease [CAD]) or angiography (obstructive CAD).*		
Objectives and Endpoints – added text to primary endpoint Modified to: "Incidence of DLTs *and frequency of AEs*"	Added text to align with Phase 1b	Section 1.1 Study Synopsis
Study Design – added text Modified to: "This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll *approximately 69 subjects* 18 to 75 years (inclusive) of age with dyslipidemias and increased levels of LDL-C or triglycerides with ASCVD that are refractory or intolerant to statin treatment. *Phase 1a – Dose Escalation* *When the DLT evaluation period ends for the last subject enrolled at each escalation DL, the Safety Review Committee (SRC) will review pharmacokinetic (PK)/pharmacodynamic (PD) and safety data and will be responsible for making decisions regarding dose escalation or de-escalation.* *Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D). The FD will be a dose associated with optimal biological efficacy, as determined by intended decrease in ANGPTL3 and key lipid levels, with minimum toxicity.* *Phase 1b – Disease-specific Cohort Expansion* *Following completion of eLBW-based dose escalation, the sponsor will review the totality of safety and clinical activity data to delineate a flat dose (FD) for Phase 1b or anticipated RP2D. The FD will be applied to the Phase1b disease-specific expansion cohorts. Pharmacokinetics, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts. The SRC will endorse the FD. Each disease-specific cohort may enroll up to 12 subjects. Subjects may be dosed concurrently within each disease-specific cohort. An additional FD may be evaluated in each expansion cohort if the safety or activity profile of the first FD is inadequate but and will not exceed the equivalent highest dose evaluated during Phase 1a. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase 1a. Phase 1b will confirm the RP2D.* *The SRC will be responsible for endorsing the recommended phase to dose (RP2D).*" "At each DL, all AEs, include adverse events of special interest (AESI)s) *will be reviewed by the* ~~Safety Review Committee (SRC) before proceeding to the next DL. Once dose escalation is completed and a recommended~~	Added text describe the process for data review in Phase 1a and Phase 1b	Section 1.1 Study Synopsis

Change	Rationale	Affected Section(s)
~~dose has been determined, at least 3 additional subjects will be enrolled at the same DL to confirm safety and PD effect (confirmatory DL)~~ *SRC before proceeding to the next DL.*		
Study Oversight *Safety Review Committee* *Modified to: "Following completion of dose escalation and analysis of PK/PD, key lipid, and safety data in Phase 1a, the SRC will evaluate the totality of clinical data to approve a FD for evaluation in Phase 1b expansion cohorts."* *Independent* Data Safety Monitoring Board Throughout the study the DSMB will review safety from dose escalation *and disease specific cohort expansions*.	Added text to describe the responsibilities of the SRC and DSMB in Phase 1a and Phase 1b	Section 1.1 Study Synopsis
Section 1.1 Protocol Synopsis and Section 5.4.1 Dose Escalation Methodology – added 2 dose levels to Phase 1a **Proposed Starting Dose and Dose Escalation** ***Phase 1a* Dose Escalation of CTX310** **Dose Level*** | **Planned Dose Based on Estimated Lean Body Weight (mg/kg eLBW) [1]** *5* | *1.0 [3]* *6* | *1.2 [4]* *[3] Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.* *[4] Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.* Dosing within a Dose Level in Phase 1a Dose Escalation Modified to: "If ≥2 of 6 subjects experience a DLT in DLs 2, 3, 4, *5, or 6*, de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL. If ≥2 of 3 subjects experience a DLT in DLs 2, 3, 4, *5 or 6* or any optional de-escalation DL as specified in the table above, de-escalate to previous DL, or declare previous DL the MTD if 6 subjects are already tested at the previous DL. ***Phase 1b – Disease-specific Cohort Expansion*** *Following completion of eLBW-based dose escalation (Table S1), analysis of PK/PD, key lipid and safety data, and approval by the SRC, a safe and efficacious FD will be evaluated in 4 disease-specific cohort expansion cohorts. Each disease-specific cohort may enroll up to 12 subjects.* *Following endorsement from the SRC, the sponsor will declare the RP2D based on the totality of clinical data for each disease-specific cohort for future studies. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 during dose escalation may be eligible for a second dose based on predefined criteria provided in a future amendment or separate study protocol.* *Toxicities will be graded and documented according to the criteria described in the protocol.*	Added 2 dose levels to Phase 1a dose escalation and added descriptive text regarding dosing of CTX310 in Phase 1b Removed additional Phase 1a confirmatory cohort for OBD affirmation and added 2 dose levels	Section 1.1 Study Synopsis and Section 5.4.1 Dose Escalation Methodology

Change	Rationale	Affected Section(s)
Dose-limiting Toxicities: Rationale and Criteria – numbering and revision of final criteria Added text: *Any other CTCAE grade ≥3 AE, other than those listed in bullets #1-4 above, that is assessed by the investigator as related to investigational product."*	Clarification of definition of DLTs for Phase 1a	Section 1.1 Study Synopsis
Section 1.1 Study Synopsis and Section 4.1 Inclusion Criteria - Criterion 3 added criterion specific to subjects in Phase 1b with severe HTG Criterion 3 Modified: "Diagnosis of persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment. *Diagnosis of HeFH or HoFH with or without ASCVD. In the Phase 1b cohort expansion part of the study* a. *Diagnosis of severe HTG defined by elevated fasting TG levels of >500 mg/dL [5.65 mmol/L]* in subjects with ASCVD at screening, despite treatment b. *Diagnosis of mixed hyperlipidemia defined by elevated fasting TG levels of >150 mg/dL – 499 mg/dL (1.7 mmol/L – 5.6 mmol/L) with LDL-C >70 mg/dL (1.8 mmol/L), in subjects with ASCVD at screening, despite treatment."* Criterion 6 Modified: Subjects on statins must be on a stable dose *for 30 days* before screening, with no planned dose increase during the study participation.	Addition of lower weight limit and criterion specific to subjects in Phase 1b with severe HTG.	Section 1.1 Study Synopsis and Section 4.1 Inclusion Criteria
Section 1.1 Study Synopsis and Section 4.2 Exclusion Criteria delineation of criterion by applicable phase of study and addition of new criterion 29 specific to phase 1b severe HoFH. Modified*: "For Phase 1b only* *29. Treatment with evinacumab within 20 weeks prior to Day1."*	Added delineation of criterion by applicable phase of study and addition of new criterion specific to phase 1b HoFH.	Section 1.1 Study Synopsis and Section 4.2 Exclusion Criteria
Section 1.3 Schedule of Assessments, Table 1 Schedule of Assessments		
Section 1.3 Schedule of Assessments Table 1 Schedule of Assessment – new row and new footnote New row: "*History of pancreatitis at Screening (Day -42 to -1)"* Modified to: "*4. Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment will be collected. Section 8.2.2."* Subsequent footnotes renumbered.	Added table row and footnote to describe medical history requirement for subjects in Phase 1b with severe HTG	Section 1.3 Schedule of Assessments
Section 1.3 Schedule of Assessments and Section 8.1.1.1 Screening and Enrollment Revision of table footnote #1: Modified to: "*For the subject's convenience, if an assessment was performed before signing the Informed Consent Form as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe."*	Clarification of assessment to avoid unnecessary procedures.	Section 1.3 Schedule of Assessments and Section 8.1.1.1 Screening and Enrollment

Change	Rationale	Affected Section(s)
Section 1.2 Study Schema		
Section 1.2 Study Schema – addition of text specifying Phase 1a in Footnote b and Acute Safety Assessment Modified to: " ≥14-day stagger between each subject within a DL *in Phase 1a dose escalation only*. Footnote b: For each DL *during Phase 1 a dose escalation*, there will be a safety monitoring period for ≥14 days between the treatment of each subject an any subsequent subjects within the DL….."	Clarification that dose stagger applies to Phase 1a only	Section 1.2 Study Schema
Section 1.3 Schedule of Assessments, Table 1 Schedule of Assessments		
Section 1.3 Schedule of Assessments Table 1 Schedule of Assessment – new row and new footnote New row: "*History of pancreatitis at Screening (Day -42 to -1)"* Modified to: "*4. Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment will be collected. Section 8.2.2."* Subsequent footnotes renumbered.	Added table row and footnote to describe medical history requirement for subjects in Phase 1b with severe HTG	Section 1.3 Schedule of Assessments
Section 2 Introduction		
Section 2.1 Dyslipidemias – deleted redundant text Modified to: Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), *the clinical development of CTX310 is focused* on ~~the following monogenic or polygenic~~ *refractory dyslipidemias*~~, with established ASCVD~~, which encompass *HTG and/or* hypercholesterolemia *syndromes* ~~with elevated LDL-C~~ *as described in Section 2.2.1 and Section 2.1.2, respectively.*	Deletion of redundant text.	Section 2.1 Dyslipidemia
Section 2.1.1 Hypertriglyceridemia - added text *Modified: "Per the Endocrine Society Clinical Practice Guidelines and the National Cholesterol Education Program Adult Treatment Panel III, normal fasting TG levels can be defined as <150 mg/dL (1.7 mmol/L), borderline high as 150 to 199 mg/dL (1.7 to 2.3 mmol/L), high as 200 to 499 mg/dL (2.3 to 5.6 mmol/L), and very high or severe as >500 mg/dL (5.6 mmol/L).* *Hypertriglyceridemia is separated into 2 populations: secondary causes related to disease, medications, or diet, and primary genetic syndromes or susceptibility (Rygiel 2018). Elevated TG levels (>150 mg/dL [1.7 mmol/L]) are present in approximately 33% of adults in the US and are most commonly due to secondary causes rather than primary genetic syndromes (Oh and Trivette, 2020).* *Acquired causes of HTG are most commonly due to medical conditions (e.g., diabetes mellitus, metabolic syndrome, central obesity, hypothyroidism, chronic kidney disease, autoimmune disorders), medications, and diet/lifestyle (e.g., alcohol use, physical activity (Rygel 2018).* *The risk of acute pancreatitis is elevated with increasing levels of serum triglycerides. Severe hypertriglyceridemia ≥500 mg/dL is an established risk factor for acute pancreatitis (Gouno-Berthold et al, 2023). The risk of acute pancreatitis is approximately 5% for triglyceride levels >1000 mg/dL and 10% to 20% for levels >2000 mg/dL. Controlling triglyceride levels to <500 mg/dL can effectively prevent recurrences. Lipid-lowering therapies play a significant role in reducing the risk of recurrent acute pancreatitis*	*Description of the association of severe hypertriglyceridemia and increased risk of pancreatitis is relevant to Phase 1b disease-specific cohort and added objective.*	*Section 2.1.1 Hypertriglyceridemia*

Change	Rationale	Affected Section(s)
in patients with elevated TG. Although TG levels close to normal may be preferable, levels <500 mg/dL represents a safe therapeutic target for prevention of recurrences (Scherer et al, 2014)." *"Genetic causes of HTG may be characterized as multifactorial chylomicronemia syndrome (MCS) and familial chylomicronemia syndrome (FCS). MCS is a polygenic condition caused by heterozygous mutations in the lipoprotein lipase gene (LPL) or in the genes for apolipoprotein C2 (APOC2), apolipoprotein A5 (APOA5), lipase maturation factor 1 (LMF-1), or glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1 (GP1HBP1), or by multiple variants that are expressed in the presence of secondary factors (D'Erasmo et al., 2019). MCS is associated with a high CVD risk (Sarwar et al., 2007; Virani et al., 2021b) and affects approximately 1 in 250 to 1 in 800 persons (Fan et al., 2020; Laufs et al., 2020; Paquette and Bernard, 2022). TG levels range from the upper level of normal to severe (>150 to 1999 mg/dL [1.7 to 22.6 mmol/L]). FCS is a rare monogenic condition caused by the same homozygous or compound heterozygous mutations observed in MCS. As most FCS patients are either LPL-deficient or lack sufficient LPL activity, these patients will likely not benefit from ANGPTL3-directed therapies such as CTX310 and are therefore excluded from this study."* *""Given the need for a significant improvement in TG-lowering therapies, based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of circulating and hepatic TGs. "*		
Section 2.1.2 Familial Hypercholesterolemia – added text Modified: "Given the need for improvements in TG and LDL- lowering therapies and based on ANGPTL3 function, treatment with CTX310 is expected to lower levels of both LDL-C and TG."	Description added to align with global protocol	Section 2.1.2 Familial Hypercholesterolemia
Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion – added text regarding rationale for lipid cutoff for severe hypertriglyceridemia in Phase 1b disease-specific cohort expansion. Modified*: "Severe HTG, defined as TG levels of >500 mg/dL (5.65 mmol/L), is a risk factor for ASCVD and is recommended for inclusion in Phase 1b . Analyses of characteristics and prevalence of chronic conditions by TG levels have shown that these conditions and multiorgan disease were common at higher TG level, including substantially increased risk of pancreatitis associated with this condition ."*	Description of the association of severe hypertriglyceridemia and increased risk of pancreatitis is relevant to Phase 1b disease-specific cohort and added objective.	Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion
Section 2.5.5 Clinical Data – New section added with summary of clinical data from subjects treated with CTX310. Modified to: *"Preliminary data from DL 1 (0.1 mg/kg) and DL2 (0.3 mg/kg) indicate a favorable safety profile. CTX310 was well-tolerated with no serious adverse events (SAEs) or serious adverse reactions (SARs), only 1 non-serious related adverse events of special interest (AESI) of rash, no events of infusion-related reactions, clinically relevant abnormal coagulation findings, or events of increased in alanine aminotransferase (ALT) or aspartate aminotransferase (AST): ≥3 × upper limit of normal (ULN) or ≥2 × the baseline value (if baseline*	Summary and benefit-risk conclusion of available clinical data regarding the safety, tolerability, and clinical activity of CTX310.	Section 2.5.5 Clinical Activity

Change	Rationale	Affected Section(s)
ALT ≥ULN), or new malignancy, and no new signals from the safety data review or literature review. All treatment-emergent adverse events (AEs) were mild to moderate in severity. There was no treatment-emergent Grade 3 or higher AE and dose dependent liver enzyme elevations were observed. The benefit-risk profile for CTX310 remains acceptable for continued clinical development."		
Section 3 Objectives and Endpoints		
Section 3 Objectives and Endpoint and Section 11.2.3 Exploratory Endpoints– added new Exploratory Endpoint relevant to Phase 1b Modified: "*For Phase 1b only* • *Change from baseline in number of acute pancreatitis events at 12 months in subjects with severe HTG"*	Addition of exploratory endpoint regarding incidence of pancreatitis as secondary measure of clinical effect in subjects with severe HTG.	Section 3 Objectives and Endpoints, Section 11.2.3 Exploratory Endpoints
Section 5 Study Design		
Section 5.1 Investigational Plan and Section 1.1 Protocol Synopsis– added Phase 1b disease-specific cohort expansion with flat dose. Modified to: "*The study is divided into 2 parts (Figure 4); Phase 1a dose escalation followed by Phase 1b disease-specific cohort expansion. Phase 1a will delineate the potential OBD (Section 5.4.2). Phase 1b will confirm the recommended Phase 2 dose (RP2D)."* *The Phase 1a dose escalation part of this study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:* • *Multifactorial chylomicronemia syndrome (MCS).* • *Homozygous familial hypercholesterolemia (HoFH).* • *Heterozygous familial hypercholesterolemia (HeFH)* • *Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).* • *Other HTG/hypercholesterolemia syndromes of undetermined etiologies.* *The Phase 1a dose escalation will include eligible subjects, regardless of underlying dyslipidemia subtype. The majority of subjects enrolled in Phase 1a are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia and HTG. Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk.* *The Phase 1b disease-specific cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD that encompass HTG and/or hypercholesterolemia syndromes in the following disease-specific cohorts:*	Added description of Phase 1a subjects and Phase 1b disease-specific cohort expansion with flat dose of CTX310	Section 1.1 Protocol Synopsis and Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
• *Severe HTG with history of ASCVD* • *HoFH* • *HeFH* • *Mixed hyperlipidemias with history of ASCVD* *Populations with ASCVD are intended to address secondary prevention with history of prior myocardial infarction (MI) or stroke or known subclinical atherosclerosis on computed tomography (CT) calcium score (>300), CT coronary arteries (obstructive coronary artery disease [CAD]) or angiography (obstructive CAD).* ***Figure 4:*** ***Study Design*** *- new figure added* *"Following completion of eLBW-based dose escalation, the SRC will review the totality of safety and clinical activity data to delineate an OBD. The SRC will also consider approval of a range of flat dose (FD) of CTX310 based on the OBD or the highest tolerated dose tested in phase1a.* *This FD (within the recommended range) will be applied to the phase1b disease expansion cohorts. Safety and clinical activity (e.g., ANGPTL3 reduction) of CTX310 will be evaluated in each of the 4 disease specific cohorts. Each disease specific cohort may enroll up to 12 subjects. Subjects will be dosed concurrently in each disease-specific cohort. Administration of CTX310 in the cohort expansion part of the study will occur in the same manner as in Phase1a.* "At each *Phase 1a dose escalation* DL, all AEs, including adverse events of special interest (AESIs), will be reviewed by the Safety Review Committee (SRC) before proceeding to the next DL *and to Phase 1b disease-specific cohort expansion. Based on analysis of the totality of clinical data and the endorsement of the Data Safety Monitoring Board (DSMB)*, the sponsor will declare the RP2D."		
Section 5.4 Dose Escalation and Disease-Specific Cohort Expansion and Section 1.1 Study Synopsis– updated section title and edited text to accommodate addition of Phase 1b disease-specific cohort expansion. Original: "~~The Sponsor will declare the RP2D at or below the MTD, or alternatively an OBD based on the analysis of clinical data. At least 3 additional subjects will be administered CTX310 at this DL (MTD or OBD) before an RP2D is confirmed. Based on ongoing assessment of benefit and risk, the SRC may stop dose escalation before an MTD is determined Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose level based on predefined criteria provided in a future amendment.~~ Modified to: "Section 5.4 Dose Escalation *and Disease-Specific Cohort Expansion*"	Section title and text were edited to accommodate addition of Phase 1b disease-specific cohort expansion	Section 1.1 Study Synopsis and Section 5.4 Dose Escalation and Disease-Specific Cohort Expansion
Section 5.4.1 Doses Escalation Methodology – revised text ~~Based on ongoing assessment of benefit and risk, the SRC may stop dose escalation before an MTD is determined.~~	Section edited in alignment with the responsibilities of the SRC	Section 5.4.1 Dose Escalation Methodology
Section 5.4.2 Optimal Biologic Dose Definition – updated section title and edited text to remove MTD Original: "~~Maximum Tolerated Dose/Optimum~~ Optimal Biologic Dose Definition	Section title and text were edited to remove definition of MTD as it is no	Section 5.4.2 Optimal Biologic Dose Definition

Change	Rationale	Affected Section(s)
~~The MTD is the highest dose for which DLTs are observed in fewer than 2 of 6 subject. An MTD may not be determined in this study~~. The OBD is the lowest dose associated with biological efficacy, as determined by intended decrease in ANGPTL3 levels with minimum toxicity."	longer applicable in the study.	
Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions During Phase1a and Phase 1b– updated section header and bulleted definition and Section 1.1 Protocol Synopsis Modified to: 'Dose-limiting Toxicity Rationale and Definitions *During Phase1a and Phase 1b"* Bullets were numbered. Bullet 1 was moved to Bullet 6: Any *other* CTCAE grade ≥3 AE, *other than those listed in bullets #1-5 above,* that is assessed by the investigator as related to investigational product."	Updated section header to specify applicability to Phase1a and Phase 1b. Modified DLT definition to specify criterion applicable to clinical (i.e., non-laboratory results)	Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions During Phase1a Dose Escalation, and Section 1.1 Protocol Synopsis and Section 1.1 Protocol Synopsis
Section 5.4.4 Disease-specific Cohort Expansion – new section Modified to: "*Following completion of eLBW-based dose escalation (Table 2), analysis of PK/PD and safety data, and approval by the SRC, a FD of CTX310 may be evaluated in a Phase 1b cohort expansion in 4 disease-specific cohorts.* *The SRC will endorse the RP2D based on the review of the totality of data including clinical activity and safety of the FD for disease-specific groups. Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future protocol amendment or separate study protocol."* Subsequent sections were renumbered as necessary.	Added new section to describe the dose determination necessary to accommodate addition of Phase1b disease-specific cohort expansion	Section 5.4.4 Disease-specific Cohort Expansion
Section 5.5.1 Dose Escalation in Phase 1a – previous section (CTX310 Dose Rationale) edited to provide discrete description of dose escalation. Modified to: *"The clinical study with CTX310 will utilize the same LNP formulation used in the CTX310 nonclinical NHP studies, allowing direct correlations between NHP dose and the expected safety and efficacy in human subjects. As CTX310 is expected to primarily distribute to the liver and not throughout the body, a dose escalation schema is based on eLBW, which takes into account sex and height rather than total body weight alone, and, therefore, was selected to reduce the risk of overdosing obese and overweight subjects. Clinical exposure data available to date indicate that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Based on available clinical CTX310 PK from dose levels 1 through 3 (n=9) (data on file), the mean dose normalized AUC0-Tlast of* [redacted] *and* [redacted] *was 3,803,333 ± 1,933,384 and 825,000 ± 241,886 (hr*ng/mL)/(mg/kg), respectively. This exposure is approximately the same as the mean dose normalized AUC0-Tlast in the pivotal NHP GLP Toxicity study* [redacted] *3,158,889 ± 990,153;* [redacted] *934,000 ± 30,199 (hr*ng/mL)/(mg/kg); Study 3359-006). This exposure data indicates that the allometric scaling from NHPs to humans may be more appropriately calculated on a mg/kg basis, and not*	Previous Section 5.5 (CTX310 Dose Rationale) edited to provide discrete description of dose escalation in Phase 1a.	Section 5.5.1 Dose Escalation in Phase 1a

Change	Rationale	Affected Section(s)
body surface area scaling, which would further increase the anticipated safety margins by 3.1-fold. Thus, scaling between NHPs and humans may support a human equivalent NOAEL of 1.0 mg/kg and toleration of 2.0 mg/kg. With gradual increments in DLs, dose escalation is expected to proceed from 0.1 to 1.2 mg/kg. Dose escalation decisions will be made in conjunction with the investigators and SRC based on the totality of safety, tolerability, and activity data."		
Section 5.5.2 Disease-specific Cohort Expansion with Flat Dose in Phase 1b – previous section (CTX310 Dose Rationale) edited to provide discrete description of dose rationale in Phase 1b Modified to: "*Due to the primary distribution of the LNP-mediated CTX310 delivery to the liver, evaluation of safety and clinical activity of FD of CTX310 is considered appropriate and consistent with other liver-targeted genetic medicines that are administered at a FD (Bakris et al., 2024; Coelho et al., 2023; Desai et al., 2023; Young et al., Fontana et al., 2024, NCT06128629, Rat et al., 2020; Srivastava et al., 2023). Earlier siRNAs (givosiran, lumasiran) initially relied on weight-based dosing (Balwani et al., 2020; Garrelfs et al., 2021) with little difference in efficacy noted regardless of weight. Similarly, in vivo gene editors that target hepatocytes using LNP vehicles likewise began development with weight-based dosing (Gillmore et al., 2021), but transitioned to flat-dosing in later phases (NCT06128629) and with newer targets (Longhurst et al., 2024). A similar strategy is planned for Phase 1b, where PK, safety (liver function tests [LFTs]) and clinical activity parameters (lowering of ANGPTL3 and key lipids [triglyceride and low-density lipoprotein [LDL]) will be used to model an efficacious and safe FD which will be evaluated in each of the 4 disease specific cohorts."*	Previous Section 5.5 (CTX310 Dose Rationale) edited to provide discrete description of dose rationale in Phase 1b	Section 5.5.2 Disease-specific Cohort Expansion with Flat Dose in Phase 1b

Change	Rationale	Affected Section(s)
Section 6 Study Treatment		
Section 6.1 Administration of CTX310 Revision of duration of infusion Modified: Subjects will receive a single IV infusion ~~within a 1-hour period~~ on Day 1, administered under medical supervision during inpatient hospitalization.	To improve patient safety during infusion, the time allowed for infusion has been extended, as outlined in detail in the Pharmacy Manual.	Section 6.1 Administration of CTX310
Section 6.1.1 Pre-Infusion Prophylaxis – revised Added another prophylaxis regimen on Day -1, the evening before CTX310 infusion on Day 1. Added text: *On Day -1, i.e., the evening prior to CTX310 administration on Day 1, an infusion prophylaxis regimen will be administered to subjects as follows:* • *Oral steroid (e.g., dexamethasone approximately 10 mg or equivalent)* Modified the following text: *On Day 1,* within 1 to 2 hours prior to the administration of study drug, an infusion prophylaxis regimen will be administered to subjects ~~The regimen consists of the following~~ *as follows*: • *Optional oral paracetamol (500 mg to 1000 mg)*	To align with global protocol and improved precautionary measures	Section 6.1.1 Pre-infusion Prophylaxis
Section 6.5.2 Prohibited Medication – added prohibited medication necessary to accommodate Phase 1b disease-specific cohort in HoFH. Modified to: "*Phase 1b only: Evinacumab within 20 weeks prior to Day 1 for subjects.*"	Added prohibited medication necessary to accommodate Phase 1b disease-specific cohort.	Section 6.5.2 Prohibited Medications
Section 7.1 General Guidance		
Section 7.1.1 Infusion-Related Reactions – text revised to provide additional background information. Modified to*: Infusion-related reactions have been reported with LNP utilizing treatments, occurring in up to 19% of patients receiving patisiran (Moghimi and Simberg 2022) . Infusion-related reactions can be either allergic reactions to foreign particles or non-immune mediated reactions. Most IRRs are mild and typically develop within minutes to several hours of initiation of the drug infusion, although symptoms may be delayed for up to 24 hours. IRRs may affect any organ system in the body. While most reactions are mild in severity, severe or fatal reactions can occur. The most common signs and symptoms of IRR are fever, chills, flushing, itching, alterations in heart rate (including tachycardia) or blood pressure (including hypotension), dyspnea, chest discomfort, back pain or abdominal pain, nausea, vomiting, diarrhea, and various types of skin rashes.*	Revised to provide additional mechanistic background information.	Section 7.1.1 Infusion-related Reactions

Change	Rationale	Affected Section(s)
In the event of an *acute* IRR, the infusion of study drug will be slowed or stopped, and the subject closely monitored until resolution of the reaction…. *Delayed IRR (>12 hours) are usually immune-mediated and respond best to corticosteroid treatment (e.g., methylprednisolone).*		
Section 8 Study Procedures		
Section 8.1.1.5 Long-term Follow-up – modified to include pancreatitis and CV events Modified to: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310 and either discontinue prior to 12 months or complete this study will be asked to participate in a separate LTFU study *for up to 15 years post infusion* to assess long term safety, durability, and *the occurrence of any clinical adverse event, including pancreatitis event* and cardiovascular events. "	Clarified specific events for inclusion in long-term follow-up.	Section 8.1.1.5 Long-term Follow-up
Section 8.1.4 Subject Withdrawal or Discontinuation – added text with explanation of long-term follow-up study. Modified to: "*Subjects who withdraw completely from this study will be asked to participate in the separate long-term follow-up study if the long-term follow-up study has been approved at the investigative site. As CTX310 is not a continuously dosed investigational product, withdrawal from the study due to an AE is not applicable.*"	Added text with explanation of long-term follow-up study.	Section 8.1.4 Subject Withdrawal or Discontinuation
Section 8.2.2 Demographics and Medical History – added medical history requirement specific to subjects in Phase 1b with severe HTG. Modified to: "*Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment will be collected".*	Added medical history requirement specific to subjects in Phase 1b with severe HTG.	Section 8.2.2 Demographics and Medical History
Section 9 Safety, Adverse Events and Study Oversight		
Section 9.5 Adverse Event Causality – removal of text ~~**Possibly Related:** There is some evidence to suggest a causal relationship between the study treatment or procedure and the AE, but alternative causes also exist.~~ ~~If the relationship between the SAE and the investigational product is determined to be "possible," a rationale for the assessment must be provided by the reporting investigator.~~	Deletion of "Possibly Related" to simplify categorization and improve clarity.	Section 9.5 Adverse Event Causality
Section 9.9 Medication Errors and Overdose – added new section Modified to: "*An overdose or medication error along with any associated adverse event, regardless of seriousness, should be reported to the sponsor on the provided SAE/AESI form within 24 hours of awareness of the medication error/overdose or associated adverse event.*" Subsequent sections were renumbered as necessary.	In alignment with regulatory requirements and protocol template	Section 9.9 Medication Errors and Overdose
Section 9.10 Pregnancy – added text Modified to: "All pregnancies will be followed through outcome *and the infant will be followed at birth and at 1 year, provided informed consent is obtained.*	Revised in alignment with regulatory requirements	Section 9.10 Pregnancy

Change	Rationale	Affected Section(s)
Any congenital anomalies and birth defects in the infant should be reported as an SAE. Additionally, any CTX310-related malignancy or death in the infant during the 12-month follow-up period should also be reported as an SAE."		
Section 11 Statistical Analyses		
Section 11.3 Analysis Sets – modified description of DLT evaluable set Modified to: The DLT evaluable set includes all subjects *during dose escalation part* who receive CTX310 infusion and complete the DLT evaluation period or discontinue early after experiencing a DLT."	Modified to provide distinction between Phase 1a and Phase 1b	Section 11.3 Analysis Sets
Section 11.4 Sample Size – updated text to accommodate addition of Phase1b disease-specific cohort expansion. Modified to: "The sample size of the study will be approximately *69* subjects. *This will include approximately 21 subjects in Phase 1a dose escalation and 24 in the phase 1b disease-specific cohort expansion. In Phase 1b, there will be approximately up to 12 subjects in each cohort."*	Updated sample size required to accommodate addition of Phase 1b disease-specific cohort expansion.	Section 11.4 Sample Size and Section 1.1 Protocol Synopsis Number of Subjects
Section 11.6.5 Exploratory Analysis of Pancreatitis Event – new section *"The annualized event rate of pancreatitis after CTX310 will be estimated using Poisson model and compared to baseline to assess the effect of CTX310 on pancreatitis events."* Subsequent sections renumbered.	New section to align with new exploratory endpoint.	Section 11.6.5 Exploratory Analysis of Pancreatitis Event
General		
Footnotes were updated based on additions and deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Minor editorial and formatting changes for consistency were made throughout, as applicable.		

ApoB: apolipoprotein B; AE: adverse event: AESI: adverse event of special interest; ASCVD: atherosclerotic cardiovascular disease; DL: dose level; eLBW: estimated lean body weight; FD: flat dose; GLP: Good Laboratory Practice; HDL-C: high density lipoprotein – cholesterol; HeFH: heterozygous hypercholesterolemia; HoFH: homozygous hypercholesterolemia; HTG: hypertriglyceridemia; LDL-C: low-density lipoprotein-cholesterol; LNP: lipid nanoparticles; MTD: maximum tolerated dose; NHP: non-human primate; NOAEL: no observed adverse effect level; OBD; optimal biological dose; PD: pharmacodynamic; PK: pharmacokinetic; RP2D: recommended phase 2 dose; SAE: serious adverse event; siRNA: small-interfering ribonucleic acid; SRC: Safety Review Committee; TG: triglycerides;

14.4.2. Summary of Changes to UK Specific Protocol Version 5.1

Change	Rationale	Affected Section(s)
Changes specific to Section 1.1 Protocol Synopsis (identical changes in the synopsis and the body of the protocol, Sections 2 through 14, are captured in chronological order by protocol body section level heading)		
Section 1.3 Schedule of Assessment - Addition of cardiac biomarker testing on Day 7. 30, and 90	During a non-clinical rat study, an elevation of creatine kinase was observed. Therefore, cardiac biomarker assessment was added on Day 7, 30 and 90 post infusion.	Section 1.3 Schedule of Assessments
Section 2 Introduction		
Section 2.1.1 Hypertriglyceridemia – removal of description of MCS and FCS, and overall HTG This level 3 numbering now applies to familial hypercholesterolemia	Removed to align with comments from MHRA regarding the description of the study population.	Removed Section 2.1.1 Hypertriglyceridemia This level 3 numbering now applies to familial hypercholesterolemia
Section 2.5.2 Rationale for the Study Population – revision of statement regarding contraception. Original: "Due to the unknown risk of on-target editing in tissues other than the liver… and male subjects enrolled in this study must agree to use ~~highly~~ effective ~~method(s)~~ of contraception from study consent through 12 months after CTX310 infusion……" Modified to: "Due to the unknown risk of on-target editing in tissues other than the liver… and male subjects enrolled in this study must agree to use *acceptable effective* contraception from study consent through 12 months after CTX310 infusion……	As per comment from MHRA, the language regarding contraception for sperm producing males was made consistent with inclusion criterion.	Section 2.5.2 Rationale for the Study Population
Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion Original:" Per AHA and CCS guidelines (Grundy et al., 2019; Pearson et al., 2021) , subjects at high risk of CVD (i.e., LDL-C ≥100 mg/dL (2.6 mmol/L) ~~for subjects without ASCVD or ≥70 mg/dL (1.8 mmol/L) for subjects~~ with established ASCVD) are recommended for this study. ~~Similarly, subjects with non-HDL-C levels of ≥160 mg/dL (4.1 mmol/L) or ApoB levels of ≥100 mg/dL (2.6 mmol/L), identified as at high risk of CVD per CCS guidelines (Pearson et al., 2021) are also recommended for this study.~~	As per comment from MHRA, the language describing the lipid cutoff values was modified to align with the description of the study population and primary objective .	Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion

Change	Rationale	Affected Section(s)
~~Triglyceride levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al., 2020; Watts, et al. 2021) and are recommended for inclusion in this study."~~ ~~Modified to: "Per AHA and CCS guidelines , subjects at high risk of cardiovascular events (i.e., LDL-C ≥70 mg/dL [1.8 mmol/L]) and with established ASCVD are recommended for this study.~~" Modified to: Per AHA and CCS guidelines (Grundy et al., 2019; Pearson et al., 2021) , subjects at high risk of cardiovascular events (i.e., LDL-C ≥70 mg/dL [1.8 mmol/L]) and with *established* ASCVD are recommended for this study."		
Section 3 Study Objectives and Endpoints		
Section 3 Study Objectives and Endpoints – revision of primary objective. Original: "To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of ~~TG and/or non-HDL-C and/or ApoB and/or~~ LDL-C, and to determine the recommended Phase 2 dose." Modified to: "To evaluate the safety and tolerability of a single ascending dose of CTX310 in subjects with refractory dyslipidemias with elevated levels of LDL-C, and to determine the recommended Phase 2 dose."	As per comment from MHRA, the primary objective was modified to align with the description of the study population.	Section 3 Objectives and Endpoints and Section 1.1 Study Synopsis, and Section 11.1 Study Objectives and Hypotheses
Section 4 Subject Eligibility		
Section 4.1 Inclusion Criteria – revision of Criterion 3 and 4 Original Criterion #3 Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of TG (>150 mg/dL [1.7 mmol/L]) and/or LDL-C (>100 mg/dL [2.6 mmol/L]; >70 mg/dL [1.8 mmol/L] for subjects in ASCVD) and/or non–HDL-C (>160 mg/dL [4.1 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L]) at screening, despite treatment. Modified Criterion #3 Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of LDL-C >70 mg/dL	Revision was made in response to a request received from the MHRA to align with description of the study population.	Section 4.1 Inclusion Criteria, Section 1.1 Study Synopsis, Section 2.5.3, Rationale for Lipid Cutoff Values for Inclusion, and Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
[1.8 mmol/L] in subjects *with* ASCVD at screening, despite treatment. Original Criterion #4 Subject lipid levels must be refractory to the maximal intensity or MTDs of standard of care lines of lipid-lowering therapies or combinations where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least 12 weeks prior to screening. Modified Criterion #4 The lipid levels must be refractory to the *MTDs* of *statins* for at least *6 months* prior to screening or the subject must be intolerant to statins. Provided rationale in Section 2.5.3 for the update to inclusion criterion #4. *The LDL-C levels must be high, in spite of continued use of MTD of statins (as monotherapy or in combination with other lipid lowering agents) for at least 6 months (refractory), to ensure that the high-risk group is included in the study. Subjects who are intolerant to statins constitute another high-risk group due to limited access to other available treatments.* Original Criterion #6 Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation. Modified Criterion #6 Subjects on *statins* must be on a stable dose before screening, with no planned dose increase during the study participation.		
Section 4.2 Exclusion Criteria – revision of Criteria 1,2, 3, 6, and 9 Original Exclusion Criterion #1	Revision was made in response to a request received from the MHRA to revise	Section 4.1 Inclusion Criteria, Section 1.1 Study Synopsis, and Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
1. ~~Subjects with FCS with <5% LPL activity, as documented in the medical history. If LPL activity testing is not documented, the subject with FCS will be excluded.~~ Modified Exclusion Criterion #1to exclude: 1. Subjects with *chylomicronemia syndromes (e.g., familial chylomicronemia syndrome [FCS]*). Original Exclusion Criterion #2a Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >~~2~~ ×upper limit of normal (ULN), or total bilirubin value >2 × ULN… Modified Exclusion Criterion #2a Aspartate aminotransferase (AST), alanine aminotransferase (ALT) >*1.5* ×upper limit of normal (ULN), or total bilirubin value >1.5 × ULN… Original Exclusion Criterion #3 Complete blood cell count: Neutrophils <1000 cells/µL (<1.0 × 10^9/L)… Modified Exclusion Criterion #3 Complete blood cell count: Neutrophils <*1500 cells/µL (<1.5 × 10^9/L)*… Original Exclusion Criterion #6 Inadequate diabetes control, with glycosylated hemoglobin >~~9~~%. Modified Exclusion Criterion #6 Inadequate diabetes control, with glycosylated hemoglobin *(HbA1c)* ≥*8*%. Original Exclusion Criterion #9 Uncontrolled or untreated thyroid disease (thyroid stimulating hormone <0.1 mIU/L or >10 mIU/L). Modified Exclusion Criterion #9 Uncontrolled or untreated thyroid disease (thyroid stimulating hormone *0.5 to 5.0 mIU/L is the usual window of "controlled" thyroid function*).	The study population to not include subjects with HTG/chylomicronemia syndromes . The exclusion criterion "total bilirubin value >2 × ULN" to > 1.5 × ULN as a level of > 2 × ULN may indicate a clinically significant level of liver injury already present ("Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation. 2009. FDA). The exclusion criterion that relates to neutrophil count to <1.5 × 10^9/L, as a level below that indicates a substantial increase in the risk of infection, and the benefit risk balance in this first-in-human trial of experimental therapy indicates that a lower level is inappropriate. The threshold of HBa1c to ≤8% as a higher level may reflect metabolic instability, which is inappropriate for a first-in-human study for gene therapy. The eligibility criterion that addressed uncontrolled or untreated thyroid disease to lie within the range of the usual window of "controlled" thyroid function.	

Change	Rationale	Affected Section(s)
Section 5 Study Design		
Section 5.1 Investigational Plan Original: "The study population will consist of subjects 18 to 75 years (inclusive) of age who have dyslipidemias with persistently high levels of ~~triglycerides (TG) and/or non-high density lipoprotein cholesterol (non-HDL-C)~~ including low-density lipoprotein cholesterol (LDL-C) ~~and/or apolipoprotein B (Apo-B),~~ above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTDs) of ~~available lipid-lowering treatments~~, diet, and lifestyle modifications (refractory population). This Phase 1 study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with ~~or without~~ atherosclerotic cardiovascular disease (ASCVD), that encompass ~~hypertriglyceridemia (HTG) and/or~~ hypercholesterolemia syndromes: • ~~Multifactorial chylomicronemia syndrome (MCS)~~ • Homozygous familial hypercholesterolemia (HoFH) • Heterozygous familial hypercholesterolemia (HeFH) • ~~Familial chylomicronemia (FCS) (with ≥5% lipoprotein lipase [LPL] activity)~~ • Other ~~HTG/~~hypercholesterolemia syndromes of undetermined etiologies. The majority of subjects enrolled are expected to be of polygenic background due to the high prevalence of polygenic hypercholesterolemia ~~and HTG. Subjects with elevated lipids of undetermined etiology will also be eligible for enrollment based on the overall known benefit of lipid-lowering treatments in reducing cardiovascular disease (CVD) risk.~~ Subjects will be asked to continue to take their baseline lipid-lowering medications in the same doses through the study period until a significant beneficial effect (i.e., achievement of target lipid goals) of CTX310 is observed." "This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll up to 24 subjects 18 to 75 years	Based upon feedback from the MHRA, revisions were made to the description of the study population to include: a. participants with established atherosclerotic cardiovascular disease and not at LDL-C target on maximum tolerated dose of hydroxymethylglutaryl CoA reductase inhibitor (statin) b. participants with established atherosclerotic cardiovascular disease and who are statin-intolerant, or for whom a statin is contraindicated c. participants with Homozygous familial hypercholesterolaemia (HoFH) d. participants with Heterozygous familial hypercholesterolemia (HeFH)	Section 5.1 Investigational Plan, Section 1.1 Study Synopsis, Section 11.1 Study Objectives and Hypotheses

Change	Rationale	Affected Section(s)
(inclusive) of age with dyslipidemias and increased levels of ~~TG (>150 mg/dL [1.7 mmol/L]) and/or~~ LDL-C ~~(>100 mg/dL [2.6 mmol/L];~~ >70 mg/dL [1.8 mmol/L] ~~for~~ ASCVD) ~~and/or non-HDL-C (>160 mg/dL [4.2 mmol/L]) and/or ApoB (>100 mg/dL [2.6 mmol/L])~~ that are refractory to ~~indicated and available~~ treatments." Modified to: The study population will consist of subjects 18 to 75 years (inclusive) of age who have dyslipidemias with persistently high levels of *cholesterol, with high levels* of low-density lipoprotein cholesterol (LDL-C), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite diet, lifestyle modifications, and maximum tolerated doses (MTDs) (refractory population), or intolerance to statins.. This *study population* will include subjects with the following monogenic or polygenic refractory dyslipidemias, with *established* atherosclerotic cardiovascular disease (ASCVD), that encompass hypercholesterolemia syndromes: • Homozygous familial hypercholesterolemia (HoFH) • Heterozygous familial hypercholesterolemia (HeFH) • Other hypercholesterolemia syndromes of undetermined etiologies. The majority of subjects enrolled are expected to be of polygenic background due to the high *prevalence of polygenic hypercholesterolemia with high levels of LDL-C*.... Subjects will be asked to continue to take their baseline lipid-lowering medications in the same doses through the study period until a significant beneficial effect (i.e., achievement of target lipid goals) of CTX310 is observed." "This is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study that will enroll up to 24 subjects 18 to 75 years (inclusive) of age with dyslipidemias and increased levels of LDL-C >70 mg/dL [1.8 mmol/L] *with* ASCVD that are refractory to statins."		

Change	Rationale	Affected Section(s)
Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions – revision of criteria for assessment Reordered and clarified the first criterion in the definition of Dose Limiting Toxicity (DLT) in protocol Section 5.4.2 (and the synopsis) to be the last in the list of DLT criteria. This clarification was made to eliminate any redundancy with other definitions that propose to qualify an event as a DLT criterion based on a laboratory abnormality. Original: "Any CTCAE grade ≥3 AE that is assessed by the investigator as related to investigational product." Modified: "Any *other* CTCAE grade ≥3 AE, *other than those listed in bullets above*, that is assessed by the investigator as related to investigational product". Deleted DLT criterion redundant with the reordering of the first bullet Original deleted: ~~Any CTCAE grade ≥3 decrease in platelet count and is assessed by the investigator as related to investigational product~~.	Reordering and deletion of criteria to clarify and remove redundancy in definitions as requested by MHRA.	Section 5.4.3 Dose-limiting Toxicity Rationale and Definitions and Section 1.1 Protocol Synopsis
Section 9 Safety, Adverse Events and Study Oversight		
Section 9.11.1 Safety Review Committee and Added: "*Any revisions to the DLT definitions or criteria, dosing schema can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented.*" Section 9.11.2 Independent Data Safety Monitoring Board Added: *Any DSMB recommended revisions to the protocol can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented.*	Revision was made in response to a request received from the MHRA	Section 9.11.1 Safety Review Committee, Section 9.11.2 Independent Data Safety Monitoring Board, and Section 1.1 Protocol Synopsis
Section 10 Stopping Rules and Study Termination		
Section 10.1 Stopping Rules for the Study – addition of requirement for regulatory authority approval prior to implementation of changes to the protocol including restarting after a stopping rule is met.	Revision was made in response to a request received from the MHRA	Section 10.1.1 Stopping Rules for the Study and Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Modified to: "*No changes to the protocol can be implemented until the application is approved by the regulatory authority.* *If any stopping rule is met, the study can only be restarted after the amendment application that presents the data and the rationale to restart is approved by the regulatory authority."*		
General		
Protocol version and date were updated. Typographical errors were corrected, as applicable. Minor editorial and formatting changes for consistency throughout, as applicable.		
Abbreviations: AE, adverse event; ALT, alanine aminotransferase, AST, aspartate aminotransferase; CTCAE; Common Terminology Criteria for Adverse Events; DLT, dose-limiting toxicity; HbA1c, glycosylated hemoglobin; INR, International Normalized Ratio; MHRA, Medicines & Healthcare Products Regulatory Agency ; UK, United Kingdom; ULN, upper limit of normal		

14.4.3. Summary of Changes to Global Protocol V5.0, Amendment 4

The key changes to global protocol Amendment 4 (V5.0) are:

- Revision of Inclusion Criterion #1 to raise the upper age limit from ≤70 to ≤75 years to be more inclusive of the intended study population.
- Revision of Inclusion Criterion #3 to lower the limit of triglycerides to >150 mg/dL (1.7 mmol/L) to be more inclusive of the intended study population.
- Revision of Exclusion Criterion #1 to exclude subjects with familial chylomicronemia syndrome with <5% lipoprotein lipase (LPL) activity.
- Addition of new Exclusion Criterion #11 regarding severe aortic stenosis.
- Deletion of Exclusion Criterion #18 regarding use of monoclonal antibodies.
- Revision of Exclusion Criterion #25 to exclude subjects with prior malignancy within 5 years for inclusion of subjects who are in remission.
- Addition of measurement of complete blood count on Days 1, 3, 7 and 14, to support monitoring of adverse events to Table 1 Schedule of Assessments.
- Addition of option for second dose for eligible subjects once the recommended Phase 2 dose (RP2D) has been selected.
- Addition of the following DLT criteria to capture hepatotoxicity, hepatocyte damage or synthetic liver dysfunction:
  - Any CTCAE grade ≥3 elevations in ALT and AST that persist for >14 days and is assessed by the investigator as related to investigational product.
  - Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) and is assessed by the investigator as related to investigational product.
  - Any CTCAE grade ≥3 decrease in platelet count and is assessed by the investigator as related to investigational product.
- To improve alignment with expected hepatotoxicities, specified that the treatment of the next subject will be paused if any of the first 4 stopping rule criteria are met in ≥2 subjects at a dose level. Specified the duration of persistence of ALT or AST elevations ≥8×ULN.

- ≥2 subjects at a DL with ALT or AST >8 × ULN, which is confirmed and persists for >14 days.
- ≥2 subjects at a DL with ALT or AST >5 × ULN, which is confirmed and persists for >30 days.
- ≥2 subjects at a DL with ALT or AST >3× ULN (or the greater of 2× baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 × ULN persisting for >14 days.

All key changes made in the global Version 5.0 of the protocol are tabulated below.

Change	Rationale	Affected Section(s)
Changes specific to Section 1.1 Protocol Synopsis (identical changes in the synopsis and the body of the protocol, Sections 2 through 14, are captured in chronological order by protocol body section level heading)		
Section 1.3 Schedule of Assessments, Table 1 Schedule of Assessments		
Section 1.3 Schedule of Assessments – revision of headers to include Day Original: "M2, M3, M6, M9, EOS/M12" Modified to: " M2/*D60*, M3/*D90*, M6/*D180*, M9/*D270*, EOS/M12/*D360*."	Assists in alignment with electronic data capture.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessments – deletion of echocardiogram on Day 30 and EOS	Modify the requirement for additional echocardiograms to be at the discretion of the investigator.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessment – Additional measurements of CBC Original: Screening, Day 2, Day 4, Day 30, Day 180, EOS Modified to: Screening, *Day 1*, Day 2, *Day 3*, Day 4, *Week 1 Day 7*, *Week 2 Day 14,* Day 30, Day 180, EOS	Additional measurements of CBC to capture adverse events for improved subject safety.	Section 1.3 Table 1 Schedule of Assessments
Section 1.3 Schedule of Assessments; modification to footnote #5 Original: "On Day 1 vital signs should be recorded at the following time points: prior to the infusion of CTX310, every 15 (± 5 minutes) during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital." Modified: ". On Day 1 vital signs should be recorded at the following time points: *prior to pre-infusion prophylaxis,* prior to the infusion of CTX310, every 15 (± 5 minutes) during the infusion,	Addition of timepoint for improved subject safety.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital."		
Section 1.3 Schedule of Assessments; modification to footnote #7 Original: "~~Echocardiogram is required at screening.~~ Transthoracic echocardiogram will be performed at screening ~~the scheduled visits~~. See Section 8.2.6." Modified to: "Transthoracic echocardiogram *will be performed at screening*. See Section 8.2.6."	Modify the requirements for transthoracic echocardiogram to provide clarity.	Section 1.3 Table 1Schedule of Assessments
Section 1.3 Schedule of Assessments; modification to footnote #8 Original: "Electrocardiogram: See Section 8.2.7. Day 1 ECG ~~will~~ be collected prior to pre-infusion prophylaxis regimen." Modified: "Electrocardiogram: See Section 8.2.7. Day 1 ECG *can be collected within 24 hours* prior to pre-infusion prophylaxis regimen."	Modification of the timepoint for collection of ECG to provide flexibility.	Section 1.3 Table 1 Schedule of Assessments
Section 2 Introduction		
Section 2.1 Dyslipidemia and Section 1.1 Protocol Synopsis; addition of FCS Based on the multiple mechanisms of ANGPTL3 function (Section 2.2 and Section 2.5.1), the clinical development of CTX310 is focused on the following monogenic or polygenic refractory dyslipidemias, which encompass HTG and/or hypercholesterolemia syndromes: • Multifactorial chylomicronemia syndrome (MCS). • Homozygous familial hypercholesterolemia (HoFH). • Heterozygous familial hypercholesterolemia (HeFH). • *Familial chylomicronemia syndrome (FCS) (with ≥5% LPL activity).* • Other HTG and/or hypercholesterolemia syndromes of undetermined etiologies.	Modification of the list to align with exclusion criterion #1	Section 2.1 Dyslipidemia and Section 1.1 Protocol Synopsis
Section 2.1.1 Hypertriglyceridemia Original: "As most FCS patients are either LPL-deficient or lack insufficient LPL activity, these patients will likely not benefit from *ANGPTL3*-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and ~~are~~, therefore~~, excluded from this study~~. Modified to: "As most FCS patients are either LPL-deficient or lack sufficient LPL activity, these patients will likely not benefit from ANGPTL3-directed therapies such as CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity and*, therefore, only FCS patients with a documented medical history of ≥5% LPL activity will be included in this study."*	Modification of the study population description to align with exclusion criterion #1	Section 2.1.1 Hypertriglyceridemia

Change	Rationale	Affected Section(s)
Section 2.4 CTX310 – clarification of language Original: "ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; EL hydrolyzes HDL." Modified: "ANGPTL3 is an endogenous inhibitor of LPL and EL. LPL hydrolyzes TG in TG-rich lipoproteins, including VLDL and chylomicron; *EL hydrolyzes phospholipids, mainly on HDL particles*."	Expansion of definition to improve accuracy	Section 2.4 CTX310
Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion Original: "~~Although TG~~ levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al, 2020; Watts et al, 2021)~~, a higher TG level (≥300 mg/dL [3.4 mmol/L]) has been~~ recommended for inclusion in this ~~study to select for a more severely dyslipidemic patient population~~. Modified to: "*Triglyceride* levels of ≥150 mg/dL (1.7 mmol/L) are associated with increased CVD, based on an evaluation of relevant investigational studies (Rosenson et al, 2020; Watts et al, 2021) *and are recommended for inclusion in this study."*	Revised to lower the limit of acceptable triglyceride value and is more inclusive of the intended population.	Section 2.5.3 Rationale for Lipid Cutoff Values for Inclusion
Section 4 Subject Eligibility		
Section 4.1 Inclusion Criteria Criterion #1, Synopsis Study Population, Study Design and Study Eligibility, Section 5.1 Investigational Plan, Section 8.1.1 General Study Periods; extension of upper limit of age requirement. Original: "Age of ≥18 and ≤~~70~~ years at the time of signing informed consent." Modified to: "Age of ≥18 and ≤*75* years at the time of signing informed consent."	Modify the eligibility criterion to be more inclusive of upper age limit.	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis and Section 5.1 Investigational Plan
Section 4.1 Inclusion Criteria Criterion #3, Synopsis Study Population qualifying triglyceride level decreased. Original: "Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of triglycerides (>~~300~~ mg/dL [~~3.4~~ mmol/L])…." Modified to: "Subjects diagnosed with persistent dyslipidemia, defined by elevated fasting (and pre-apheresis, if applicable) levels of triglycerides (>*150* mg/dL [*1.7* mmol/L])…."	Revised to lower the limit of acceptable triglyceride value and is more inclusive of the intended population.	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 4.1 Inclusion Criterion #4, Synopsis Study Population modification of definition of 'refractory' Original: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least ~~26~~ weeks prior to screening." Modified to: "Subject lipid levels must be refractory to the *maximal intensity or* MTDs of standard of care lines of *lipid lowering therapies or combinations* where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab), for at least *12* weeks prior to screening."	Currently, no patients will be on single line of treatment before being labelled refractory but will be on combinations of lipid lowering medications. Therefore, the definition of 'refractory' has been revised to encompass all routinely available medications in maximal intensity or MTD. The time it takes to respond to individual medications was evaluated and it was concluded that 12 weeks on maximal intensity /doses of any combination of these medications will be sufficient time to gauge the response	Section 4.1 Inclusion Criteria; Section 1.1 Protocol Synopsis
Section 4.1 Inclusion Criterion #6, Synopsis Study Population Original: "Subjects on available standard of care lines of treatment, ~~including but not limited to, statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors,~~ must be on a ~~maximum tolerated and~~ stable dose ~~>30 days~~ before screening, with no planned dose increase during the study participation." Modified to: "Subjects on available standard of care lines of treatment must be on a stable dose before screening, with no planned dose increase during the study participation. "	Revised criterion to require subject be on a stable dose be on a stable dose of treatment to align with standard of care for the intended population.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria Criterion #1 Original: "Subjects with FCS as documented in the medical history." Modified to: "Subjects with FCS *with <5% LPL activity* as documented in the medical history. *If LPL activity testing is not documented, the subject with FCS will be excluded.*	As most FCS patients are either LPL-deficient or lack sufficient LPL activity, they will likely not benefit from CTX310 due to the direct mechanism of action of ANGPTL3 on LPL activity. Therefore, only FCS patients with a documented medical history of <5% LPL activity will be excluded.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria – new Criterion #11. New: "*Severe aortic stenosis (peak velocity ≥4 m/s or aortic valve area <1 cm²)*." Criterion #11 through #17 were renumbered to Criterion #12 through 18	New criterion added to improve subject safety	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria – deleted Criterion #18. Original: "~~Current use or use within 90 days from Day 1 of any monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab).~~"	Subjects on monoclonal antibody treatments are not excluded from this Phase 1 study to be more inclusive of the intended population.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 4.2 Exclusion Criteria and Synopsis Study Eligibility Exclusion Criteria Criterion #25; revised timeframe for prior malignancy Original: "Any prior or current malignancy except for the following that have been completed resected or removed: basal cell carcinoma, squamous cell carcinoma and carcinoma in situ of the cervix or breast, or myeloproliferative disorder or a significant immunodeficiency disorder." Modified to: "Any prior malignancy *within the past 5 years* or current malignancy, *(*except for the following that have been completed resected or removed: basal cell carcinoma, squamous cell carcinoma and carcinoma in situ of the cervix or breast*)*, or myeloproliferative disorder. or a significant immunodeficiency disorder."	Revised timeframe to within past 5 years for prior malignancy to be inclusive of subjects who are in remission.	Section 4.2 Exclusion Criteria and Section 1.1 Protocol Synopsis
Section 5 Study Design		
Section 5.4.1 Dose Escalation Methodology and Section 1.1 Protocol Synopsis - addition of statement referencing dose rationale. Column Modified to: "*The dose rationale based on nonclinical studies in NHPs is described below."* **Table 3 Dose Escalation of CTX310** **Dose Level*** | **Planned Dose *Based on Estimated Lean Body Weight* (mg/kg eLBW)[1]**	Addition of statement referencing Dose Rationale provided for clarity. Column header in Table 2 revised for clarity.	Section 5.4.1 Dose Escalation Methodology, Table 3 Dose Escalation of CTX310, and Section 1.1 Protocol Synopsis
Section 5.4.1 Dose Escalation Methodology and Synopsis Proposed Starting Dose and Dose Escalation Modified to: "*Once the RP2D is determined, subjects who may have received a lower dose of CTX310 may be eligible for a second dose based on predefined criteria provided in a future amendment.*"	Addition of option for second dose for eligible subjects once the dose escalation of CTX310 is complete and an RP2D demonstrating clinical benefit and acceptable safety profile is selected	Section 5.4.1 Dose Escalation Methodology and Section 1.1 Protocol Synopsis

Change	Rationale	Affected Section(s)
Section 5.4.3 and Study Synopsis Dose-limiting Toxicity Rationale and Definition Added new bullets: • *Any CTCAE grade ≥3 elevations in ALT and AST that persist for >14 days and is assessed by the investigator as related to investigational product.* • *Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) that is assessed by the investigator as related to investigational product.* • *Any CTCAE grade ≥3 decrease in platelet count that is assessed by the investigator as related to investigational product.* Original: • Any CTCAE grade ≥3 AE that is related to ~~study drug~~. • "Any CTCAE grade 3 laboratory abnormality that persists ≥7 days and is related to the ~~study drug~~. • Any CTCAE grade 4 laboratory abnormality that is related to the ~~study drug~~." Modified to: • Any CTCAE grade ≥3 AE that is *assessed by the investigator as* related to *investigational product.* • Any *other* CTCAE grade 3 laboratory abnormality that persists ≥7 days and is *assessed by the investigator as* related to the *investigational product*. • Any *other* CTCAE grade 4 laboratory abnormality that is *assessed by the investigator as* related to the *investigational product*."	Addition of new bullets and clarification of bullets regarding laboratory abnormalities to enhance subject safety and capture liver hepatotoxicity, synthetic liver dysfunction.	Section 5.4.3 Dose-limiting Toxicity Rationale and Definition; Section 1.1 Protocol Synopsis
Section 6 Study Treatment		
Section 6.5.2 Prohibited Medications; removal of monoclonal antibodies Original: "~~Monoclonal antibody treatment (except evolocumab, alirocumab, or evinacumab)~~"	Subjects on monoclonal antibody treatments are not excluded from this Phase 1 study to be more inclusive of the intended population.	Section 6.5.2 Prohibited Medications

Change	Rationale	Affected Section(s)
Section 8 Study Procedures		
Section 8.2.6 (New number) Echocardiogram -added language Original: "~~Echocardiogram is required at screening~~. A transthoracic echocardiogram for assessment of LVEF will be performed at ~~time points specified in the schedule of assessments~~ (Table 1). Additional echocardiograms may be obtained at the investigator's discretion." Modified to: "A transthoracic echocardiogram *is required at screening* for assessment of LVEF *for all subjects* (see Schedule of Assessments Table 1). Additional echocardiograms may be obtained at the investigator's discretion."	Additional guidelines to enhance subject safety and streamline procedures.	Section 8.2.6 Echocardiogram
Section 8.2.8 Laboratory Tests, Table 4 Local Laboratory Testing Original: "Urinalysis – albuminuria (dipstick) or urine ACR" Modified to: *ACR[1]" "Footnote 1. Albuminuria (dipstick) or ACR to be performed at screening as specified in the schedule of assessments (Table 1)"*	Modified for consistency of language within the program.	Section 8.2.8 Laboratory Tests Table 4 Local Laboratory Testing
Section 9 Safety, Adverse Events and Study Oversight		
Section 9.7 Adverse Event Collection; revision of text Original: "The safety-related information of all subjects ~~enrolled~~ in this study will be recorded from the time of signing the ICF until EOS; however, there are different reporting requirements for the different time periods in the study." Modified to: "The safety-related information of all subjects in this study will be recorded from the time of signing the ICF until EOS/*M12*; however, there are different reporting requirements for the different time periods in the study." Original: "If a subject does not receive CTX310 therapy ~~after enrollment~~, the AE reporting period ends. ~~30 days after last study-related treatment or procedure (e.g., pretreatment regimen, imaging)~~." Modified to: "If a subject does not receive CTX310 therapy, the AE reporting period ends."	Editorial revision for improved clarity	Section 9.7 Adverse Event Collection Period

Change	Rationale	Affected Section(s)
Section 10 Stopping Rules and Study Termination		
Section 10.1 Stopping Rules for the Study– modify language Original: "~~The occurrence of laboratory results meeting any of the criteria in Section 10.1.1 will result in immediate suspension of accrual to the study for safety reasons pending review of the data by the sponsor, SRC, and DSMB~~. Modified to: "*Administration of investigational product will be paused temporarily for safety reasons, pending review of the data by the sponsor, SRC, and DSMB, upon the occurrence of laboratory results meeting any of the criteria in Section* 10.1.1."	Revision of language to clarify temporary nature of suspension while under review.	Section 10.1 Stopping Rules for the Study
Section 10.1.1 Stopping Rules – modification of rules 1 to 3 and reordering of numbers to accommodate addition. Original: "Study accrual-will be ~~suspended~~ as described in Section 10.1 if any of the following criteria are met: 1. ALT or AST >8 × ULN, which is confirmed by repeat testing. 2. ALT or AST >5 × ULN, which is confirmed and persists for >~~4 weeks~~. 3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 persisting for >~~2 weeks~~. 4. Fatal or life-threatening AE that is due to ~~study drug~~. Modified to: *Treatment of the next subject* will be *paused* as described in Section 10.1 if any of the following criteria are met: 1. *≥2 subjects at a DL with* ALT or AST >8 × ULN, which is confirmed *and persists for >14 days*. 2. *≥2 subjects at a DL with* ALT or AST >5 × ULN, which is confirmed and persists for *>30 days.* 3. *≥2 subjects at a DL with* ALT or AST >3× ULN (or the greater of 2× baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 × *ULN* persisting for *>14 days*. 4. Fatal or life-threatening AE that is due to *investigational product.*	To improve alignment with expected hepatotoxicities, specified that the treatment of the next subject will be paused if the first 3 stopping rule criteria are met in ≥2 subjects at a dose level. Added the duration of persistence of ALT or AST elevations ≥8×ULN for the first rule.	Section 10.1.1 Stopping Rules for the Study

Change	Rationale	Affected Section(s)
Section 11 Statistical Analyses		
Section 11.3 Analysis Sets – added definition of DLT Evaluation Set and modification of definition of Full Analysis Set New: "*The DLT evaluable set includes all subjects who received CTX310 infusion and complete the DLT evaluation period or discontinue after experiencing a DLT. The DLT period will begin with CTX310 infusion and last for 30 days, or beyond for improvement or resolution of the signs and symptoms of a potential DLT. The DLT evaluable set will be used for dose escalation decisions.*" Original: "The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment ~~or discontinue earlier~~. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL." Modified to: "The full analysis set (FAS) is a subset of the safety analysis set that includes subjects who receive CTX310 infusion and have at least 1 post-baseline lipid assessment. The efficacy analyses will be performed based on the FAS. Subjects in the FAS will be classified by received CTX310 DL."	Addition of the definition of the DLT evaluable set needed to support a complete data analysis.	Section 11.3 Analysis Sets
Section 14 Appendix: Definition and Supporting Operational Details		
Section 14.2 Contraception Requirements – added text New: "*Note: Male condoms without spermicide may be used with another acceptable method of female contraception listed below (see acceptable contraceptive methods for female partners of male subjects).*"	Added text for clarification	Section 14.2 Contraception Requirement
Section 14.2 Contraception Requirements – revision of text Original: "Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of ~~mobilization~~ and 1 barrier method of contraception." Modified to: "Vasectomy (with a negative sperm post-vasectomy semen analysis) at least 6 months before start of *enrollment* and 1 barrier method of contraception."	Clarification of timepoint	Section 14.2 Contraception Requirement

Change	Rationale	Affected Section(s)
General		
The term "preclinical" was replaced with "nonclinical' throughout the document for consistency. Footnotes were updated based on additions and deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Minor editorial and formatting changes for consistency throughout, as applicable.		

ALT: alanine aminotransferase; AST; aspartate aminotransferase; CBC: complete blood count; CRF: case report form; CTCAE: Common Terminology Criteria for Adverse Events; DSMB: Data Safety Monitoring Board; DL: dose level; eLBW: estimated lean body weight; EOS: end of study; ICF: informed consent form; INR: international normalized ratio; M12: month 12; MRE: magnetic resonance elastography; MRI: magnetic resonance imaging; NHP: non-human primates;mPDFF: protein density fat fraction; SRC: Safety Review Committee; ULN: upper limit of normal.

14.4.4. Summary of Changes to Global Protocol V4.0, Amendment 3

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments: Removed the table row and Footnote #7 for Cardiac Stress Imaging ~~*"Cardiac stress imaging with echocardiography or rMPI (SPECT or PET) will be performed at screening. See Section 8.2.6 for details."*~~ The Footnote numbering has been adjusted.	The cardiac stress imaging was added in the prior amendment by the sponsor as an additional assessment to enable assessment of clinically relevant ASCVD during enrollment. However, this assessment may impact inclusion of the intended study population in this clinical study. Other cardiac assessments, e.g., echocardiogram and ECG assessments have already been included for decision regarding eligibility	Section 1.3, Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #1 and Section 8.1.1.1 Screening and Enrollment: removed mention of cardiac stress imaging Modification shown in strikethrough: "All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, ~~cardiac stress imaging,~~ and infectious disease markers (if these procedures were performed within 60 days prior to infusion)."		Section 1.3, Table 1 Schedule of Assessments, Section 8.1.1.1.
Protocol Synopsis and Section 4.2 Exclusion Criteria: Removed Exclusion Criterion #28. *"*~~*28.Abnormal finding of myocardial ischemia on cardiac stress imaging (stress echocardiogram or radionuclide myocardial perfusion imaging [rMPI]) at screening. If results are inconclusive, findings should be discussed with the Safety Review Committee (SRC) prior to enrollment*~~*."* The downstream Exclusion Criterion numbering has been adjusted and the total number of exclusion criteria has changed from 29 to 28.		Section 1.1 Protocol Synopsis; Section 4.2 Exclusion Criteria
Section 8.2.6 Cardiac Stress Imaging: removed and downstream level 3 subheadings have been adjusted accordingly. *"*~~*8.2.6 Cardiac Stress Imaging*~~ ~~*A stress echocardiogram or rMPI (using single-photon emission computed tomography [SPECT] or positron emission tomography [PET]) will be performed before CTX310 administration at screening as described in the schedule of assessments (Table 1). Exercise or a pharmacologic agent may be used to induce stress conditions. Standard local regulations will be used to image acquisition and interpretation. During screening, if the results of stress echocardiogram or rMPI are not clearly interpretable for a subject, the sponsor may approach the SRC for a decision regarding eligibility*~~*."*		Section 8.2.6 Cardiac Stress Imaging. Downstream level 3 section heading numberings updated

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments current Footnote #7 for Echocardiogram and Section 8.2.7 (renumbered to 8.2.6) Echocardiogram: removed mention of cardiac stress imaging. Modification shown in strikethrough: Echocardiogram is required at screening. A transthoracic echocardiogram (for assessment of LVEF) will be performed at the time points specified in the schedule of assessments (Table 1). ~~unless done as part of cardiac stress imaging. The LVEF obtained during the resting portion of a stress echocardiogram (Section 8.2.6) may be used for screening purposes~~. Additional echocardiograms may be obtained at the investigator's discretion.		Section 1.3, Table 1 Schedule of Assessments, Section 8.2.6 Echocardiogram (section renumbered)
Schedule of Assessments: added a new row: for cardiac biomarker test prior to infusion of CTX310 and added reference to Footnotes #11 and #12 Section 6.1 Administration of CTX310 *Prior to administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor (see Table 1).* Also replicated this statement in Schedule of Assessment Footnote #12 Section 8.2.8 Laboratory Tests and Table 4 Local Laboratory Tests *Cardiac biomarker* | *Troponin I or T*	Tests for elevation of cardiac biomarker as a safety measure prior to CTX310 administration	Section 1.3 Table 1 Schedule of Assessments, Section 6.1 Administration of CTX310, Section 8.2.8 Laboratory Tests Table 4 Local Laboratory Tests
Modified Inclusion Criterion #4 to remove the ANGPTL3 inhibitor, evinacumab, from the list. Deletion is shown in Strikethrough in modified Inclusion Criterion #4: Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants, monoclonal antibodies to PCSK9 (alirocumab or evolocumab) ~~or ANGPTL3 (evinacumab~~), for at least 26 weeks prior to screening.	Evinacumab (Evkeeza®) is not available or has only recently been approved in some countries (e.g., Health Canada approval on 25 September 2023) and subjects may not be able to meet the criteria of refractoriness to maximum tolerated dose (MTD) for at least 26 weeks prior to screening.	Section1.1 Synopsis – Inclusion Criteria; Section 4.1

Change	Rationale	Affected Section(s)
Modified Exclusion Criterion 3 as shown in italics: Complete blood count: neutrophils <1000 cells/μL (1.0×10^9/L); lymphocytes <500 cells/μL (0.5×10^9/L); hemoglobin <11 g/dL (~~6.83mmol~~ *110 g/L*) for males, <10 g/dL (~~6.21 mmol~~ *100 g/L*) for females; or platelet count <100,000/μL (100×10^9/L).	Replaced unit of measurement for hemoglobin in mmol with standard international (SI) unit.	Section 1.1 Protocol Synopsis; Section 4.2
Updated current Footnote #18 in the Schedule of Assessments (Table 1) and applied clarifications in Section 8.4.1 for consistency. Original: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, D2, D3, and D4 (48-, 72-, and 96-hours post-infusion time points, respectively), a single sample will be collected (Section 8.4.1)." Modified to: "PK testing: *See Section 8.4.1*: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 *(±5 min)*, 2 *(±5 min)*, and 7 *(±15 min)* hours after completion of CTX310 infusion. On D2, D3, and D4 (*24 [±2 hours]-, 48 [±2 hours] -, and 72 [±2 hours]* -hours post-infusion time points, respectively), a single sample will be collected. A single sample will also be collected for all other scheduled timepoints." Updated Section 8.4.1 to align with the Schedule of Assessments and consolidate the windows for timepoints in Table 1 Footnote #18. Original: "On Day 1, samples will be collected at prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after the completion of CTX310 infusion. For all other time points, D2, D3, and D4 (48, 72, and 96 hours post-infusion time points, respectively), a single sample will be collected." Modified to: On Day 1, samples will be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at the scheduled time points after the completion of CTX310 infusion, *as described in the schedule of assessments (Table 1)*. For all other time points specified *in the schedule of assessments (Table 1)*, including D2, D3, D4, a single sample will be collected as described.	Added windows for timing of PK sample collection and corrected the hours of the timepoints corresponding to D2, D3, and D4.	Section 1.3 Table 1 Schedule of Assessments, Section 8.4.1

Change	Rationale	Affected Section(s)
Updated current Footnote # 22 in the Schedule of Assessments (Table 1). Original: "Serum samples for exploratory biomarker assessments (e.g., cytokines): On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, a single sample will be collected (Section 8.4.3.3)." Updated Footnote #22 Modified to: "Serum samples for exploratory biomarker assessments (e.g., cytokines*): See Section 8.4.3.3:* On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 *(±5 min),* 2 *(±5 min)*, and 7 *(±15 min)* hours after completion of CTX310 infusion. *On D2 and D3 (24 [±2 hours]-, and 48 [±2 hours] -hours post-infusion time points, respectively)*, a single sample will be collected."	Added windows for timing of sample collection and corrected the hours of the timepoints corresponding to D2 and D3.	Section 1.3 Table 1 Schedule of Assessments

Glossary of Terms in the appendix was updated. Section numbering, footnotes were updated based on deletions. Protocol version and date were updated. Typographical errors were corrected, as applicable. Editorial changes were applied for clarification, as needed.

14.4.5. Summary of Changes to Global Protocol V3.0, Amendment 2

Change	Rationale	Affected Section(s)
Schedule of Assessments		
Table 1 Schedule of Assessments Footnote #1; added text "*Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, cardiac stress imaging, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).*"	Addition of text to the schedule of assessment Footnote 1 for clarity	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #3; clarified text Original: "All participants who receive an infusion of CTX310, including those who terminate the study prior to 12 months, will be asked at the EOS visit to sign an informed consent for rollover into a separate long-term follow-up study for up to 15 years post-infusion (Section 8.1.1.5)." Modified to: "All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months and those who complete the study, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion (Section 8.1.1.5)."	Clarified that the subjects who discontinue the study prior to M12 will be asked to consent to roll over into a separate long-term follow-up study for up to 15 years after post-infusion.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #4; added D1 Original: "Complete physical exam required at screening, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3). Modified to: "Complete physical exam required at screening, *D1*, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (Section 8.2.3)."	Added a full physical exam on Day 1	Section 1.3 Schedule of Assessments, Table 1 Section 8.2.3 Physical Examination, Height and Weight
Table 1 Schedule of Assessments Footnote #5; added text. Original: "On Day 1 vital signs should be recorded at the following time points: prior to the infusion of CTX310, every 15 minutes during the infusion, at 1, 2, and 5 hours after the end of the infusion, and then every 8 hours until discharge from the hospital." Modified: "On Day 1 vital signs should be recorded at the following time points: prior to the infusion of CTX310, every 15 ± *5* minutes during the infusion, at 1, 2, *3, 6 hours (± 15 minutes), and 8* hours (± *30 minutes)* after the end of the infusion, and then every 8 hours (± *30 minutes)* until discharge from the hospital."	Increased the number of monitoring time points and added windows for monitoring for vital signs post-infusion on Day 1 and Day 2.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments: Added a new Footnote #7 for Cardiac Stress Imaging "Cardiac *stress imaging with echocardiography or rMPI (SPECT or PET) will be performed at screening.* See Section 8.2.6 for details." The downstream Footnote numberings have been adjusted (e.g., Original Footnote #7 has been updated to Footnote #8: : "Echocardiogram: See Section 8.2.7 for details) A new Section, Section 8.2.6, has been added for cardiac stress imaging and downstream level 3 headings have been adjusted accordingly	The cardiac stress imaging will enable assessment of clinically relevant ASCVD during enrollment.	Section 1.3 Table 1 Schedule of Assessments, Section 8.2.6 Cardiac Stress Imaging
Table 1 Schedule of Assessments Footnote #8 Original: [8]Echocardiogram: "Transthoracic echocardiogram will be performed at the scheduled visits. See Section 8.2.7 for details." Modified: [8]Echocardiogram: "Transthoracic echocardiogram will be performed at the scheduled visits. See Section 8.2.7 for details. *Required at screening unless done as part of cardiac stress imaging. The LVEF obtained during the resting portion of a stress echocardiogram (Section 8.2.6) may be used for screening purposes."*	Added text to allow for LVEF obtained during the stress echocardiogram to be used for screening purposes.	Section 1.3 Table 1 Schedule of Assessments Section 8.2.7 Echocardiogram
Table 1 Schedule of Assessments Footnote #9. Original: "ECG: See Section 8.2.7." "ECG: See Section 8.2.8. *Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen.*"	Clarification that Day 1 ECG will be collected prior to the pre-infusion prophylaxis regimen.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #14; removed D1 pregnancy test requirement. Table footnote 10 redundant text deleted. Original: "Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment. ~~Females of childbearing potential must be excluded from the study. Females with a uterus must be postmenopausal, defined by at least 12 consecutive months of amenorrhea without an alternative medical cause prior to screening, or surgically sterile (i.e., documented hysterectomy, bilateral salpingectomy, or oophorectomy at least 1 month prior to screening~~). " Modified to: "Study eligibility must be determined by the investigator and confirmed by the medical monitor (Section 8.1.1.1) prior to inpatient admission for study treatment.~~"~~	Pregnancy test at Day 1 is not required as it will be performed at screening, as per local standard, for this trial that will enroll women who meet the criteria of being non-childbearing. Redundant text deleted from Table 1 Footnote 10.	Section 1.3 Table 1 Schedule of Assessments

Change	Rationale	Affected Section(s)
Table 1 Schedule of Assessments footnote 14 revised. Original: "All females ~~enrolled in the study~~ must have a negative pregnancy test ~~within 72 hours prior to CTX310 infusion~~ (Section 8.2.8, Table 4, and Section 8.2.9)." Modified to: "*At screening*, all females must have a negative *serum* pregnancy test *performed as per local standard* (Section 8.2.9Table 4, and Section 8.2.10)."		
Table 1 Schedule of Assessments Footnote #13; clarified D1 assessment Original: "See listings of laboratory assessments (Table 4 and Table 5) for details. On Day 1 laboratory values should be performed prior to the infusion of CTX310. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to define AESI (Section 9.3) and stopping rules (Section 10.2)." Modified to: "See listings of laboratory assessments (Table 4 and Table 5) for details. Day 1 laboratory *assessments* should be performed *within 24 hours* prior to the infusion of CTX310. On all other days, serum chemistry may be repeated as required as per Section 7.1.2 to *monitor* AESI (Section 9.3) and stopping rules (Section 10.2)."	Added to provide clinical sites a flexible time window to perform local laboratory assessments prior to CTX310 infusion.	Section 1.3 Table 1 Schedule of Assessments
Table 1 Schedule of Assessments Footnote #15; clarified genetic testing for rescreen. Original: "The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.8 and Table 5 for details." Modified to (italicized text also added as new footnote 1 to Section 8.2.9 Table 5): "The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See Section 8.2.9 and Table 5 for details. *Collection of a sample for genetic testing should not be repeated during rescreening, if applicable*."	Clarification of the need for a single sample for genetic testing.	Section 1.3 Table 1 Schedule of Assessments, Section 8.2.9 Laboratory Tests Table 5 Central Testing
Table 1 Schedule of Assessments Footnote #19; clarified timing of PK assessments. Original: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points a single sample will be collected (Section 8.4.1)." Modified to: "PK testing: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1, 2, and 7 hours after completion of CTX310 infusion. For all other time points, *D2, D3, and D4 (48-, 72-, and 96-hours post-infusion time points, respectively),* a single sample will be collected (Section 8.4.1)."	Clarification of time points at with requirement for a single sample.	Section 1.3 Table 1 Schedule of Assessments Section 8.4.1 CTX310 Pharmaco-kinetic Analysis

Change	Rationale	Affected Section(s)
Introduction		
Section 2.2 ANGPTL3 Original: "~~ANGPTL3 regulates plasma TG levels by inhibiting LPL, which is the key enzyme responsible for the breakdown or hydrolysis of TG into free fatty acids (FFA) and glycerol. ANGPTL3 also inhibits endothelial lipase (EL), which is primarily responsible for the lipolysis of HDL-C. In addition to its role in regulating TG levels, mice and humans with mutant ANGPTL3 or inactivation of ANGPTL3 rapidly clear VLDL in an independent manner (Adam et al, 2020; Musunuru et al, 2010; Shimizugawa et al, 2002; Wang et al, 2015). VLDL is a precursor byproduct for the production or metabolism of LDL-C, hence its clearance limits the production of LDL-C. Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Dewey et al, 2017) . ANGPTL3 inhibition also substantially lowers ApoB levels, which has been shown to proportionally decrease CVD risk(Ference et al, 2019)~~ ." Modified to: "*ANGPTL3* regulates plasma lipid levels through inhibition of LPL and endothelial lipase (EL). Dyslipidemic mice treated with the ANGPTL3-targeting monoclonal antibody evinacumab exhibited reductions in TG, LDL-C, and HDL-C, and a significant decrease in atherosclerotic lesions (Pouwer et al, 2020). *ANGPTL3* LOF variants have been associated with decreased levels of TG, LDL-C, non-HDL-C, ApoB, and HDL-C, as well as a 41% lower risk of coronary artery disease (Dewey, et al 2017). Mechanistic studies indicate that ANGPTL3 inhibition leads to clearance of VLDL remnant particles, upstream of LDL formation, and that LDL-C lowering with ANGPTL3 inhibitors is independent of LDL receptor function (Adam et al, 2020; Reeskamp et al, 2021; Wu et al, 2020)."	Clarification of text	Section 2.2 ANGPTL3
Study Design		
Update to Investigational Plan to align with the dose escalation methodology. Section 5.1 Investigational Plan, Paragraph 7. Original: "~~Once dose escalation is completed and a RP2D has been determined, at least 3 additional participants will be enrolled at the same dose level to confirm safety and pharmacodynamic (PD) effect (confirmatory cohort)~~." Modified to: "*The sponsor will declare the RP2D at or below the MTD, or alternatively an optimum biological dose (OBD) based on the analysis of clinical data. At least 3 additional subjects will be administered CTX310 at this DL (MTD or OBD) before an RP2D is confirmed. A dose expansion cohort may be added to the protocol in a future amendment.*"	Alignment of language with Section 5.4.1 Dose Escalation Methodology for clarity.	Section 5.1 Investigational Plan

Change	Rationale	Affected Section(s)
Section 1.1 Protocol Synopsis Study Population and Section 5.1 Investigational Plan: Original: "The study population will consist of subjects 18 to 70 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride (TG) and/or non–high-density lipoprotein cholesterol (HDL-C), including low-density lipoprotein (LDL), ~~very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), and lipoprotein(a) (Lp(a)),~~ and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTD) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population)." Modified to: "The study population will consist of subjects 18 to 70 years (inclusive) of age who have dyslipidemias with persistently high levels of triglyceride(s) (TG) and/or non–high-density lipoprotein cholesterol (non-HDL-C), including low-density lipoprotein cholesterol (LDL), and/or apolipoprotein B (ApoB), above the thresholds recommended by American College of Cardiology (ACC) and American Heart Association (AHA) guidelines despite maximum tolerated doses (MTDs) of available lipid-lowering treatments, diet, and lifestyle modifications (refractory population)."	Clarification of the description of the study population.	Section 1.1, Protocol Synopsis, Section 5.1 Investigational Plan
Section 1.1 Protocol Synopsis Dose Escalation – addition of footnoted statement regarding addition of intermediate dose level(s). "* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose."	Addition of statement supporting addition of intermediate dose level(s) to support the safe execution of the trial	Section 1.1, Protocol Synopsis, Dose Escalation of CTX310 table and Dose Limiting Toxicity Assessment, Section 5.4.1 Dose Escalation Methodology Table 3 Dose Escalation of CTX310

Change	Rationale	Affected Section(s)
Study Objectives and Endpoints		
Synopsis Objectives and Endpoints, Section 3 Study Objectives and Endpoints, Section 11.1, and Section 11.2.1 Primary Endpoints. Original: "Incidence of AEs, including TEAEs, AESIs, DLTs; clinically significant laboratory abnormalities; and clinically significant abnormal vital signs" Modified to: "Incidence of ~~AEs, including TEAEs, AESIs,~~ DLTs; ~~clinically significant laboratory abnormalities; and clinically significant abnormal vital signs~~" New Secondary Objective in Synopsis and Section 3 Study Objectives and Endpoints: "*To further characterize the safety of CTX310*" New Secondary Endpoint in Synopsis, Section 3 Study Objectives and Endpoints, and Section 11.2.2.2 Secondary Safety Endpoint: "*Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs*"	Alignment of primary endpoint with the primary study objective and addition of a secondary objective and corresponding endpoint to further characterize the safety of CTX310.	Section 1.1, Synopsis Objectives and Endpoints, Section 3 Study Objectives and Endpoints, Section 11.1 Study Objectives and Hypotheses, Section 11.2.1 Primary Endpoints, Section 11.2.2.2 Secondary Safety Endpoints
Subject Eligibility		
Inclusion Criterion #4 addition of fibrates and omega-3 Original: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, icosapent ethyl, and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening." Modified to: "Subjects must be refractory to the MTDs of standard of care lines of treatment where indicated and available through routine clinical care, including statins, and/or ezetimibe, lomitapide, bempedoic acid, niacin, *fibrates, omega-3 (e.g., ethyl esters of eicosapentaenoic acid [EPA] or docosahexaenoic acid [DHA]), bile acid sequestrants,* ~~icosapent ethyl,~~ and monoclonal antibodies to proprotein convertase subtilisin/kexin type 9 (PCSK9) (alirocumab or evolocumab) or ANGPTL3 (evinacumab), for at least 26 weeks prior to screening."	Addition of fibrates and omega-3 as each is considered a standard of care	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria
Inclusion Criterion #5 removal of "homozygous familial hypercholesterolemia" Original: "Participants ~~with homozygous familial hypercholesterolemia~~ receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening." Modified to: "Subjects receiving PCSK9-targeted interfering RNA therapy (inclisiran) must be refractory to at least 365 days of exposure prior to screening."	Clarification that Inclusion Criterion #5 is not intended for subjects with homozygous familial hypercholesterolemia. This was a typographical error and does not impact any other section of the protocol.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria

Change	Rationale	Affected Section(s)
Inclusion Criterion #6 addition of "but not limited to" Original: "Subjects on available standard of care lines of treatment, including statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors, must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation." Modified to: "Subjects on available standard of care lines of treatment, including, *but not limited to* statins, and/or ezetimibe, lomitapide, bempedoic acid and/or PCSK9 and/or ANGPTL3 inhibitors, must be on a maximum tolerated and stable dose >30 days before screening, with no planned dose increase during the study participation."	Addition of text to allow for more options of standard of care.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria.
Inclusion Criterion #7 addition of "except as required by the protocol." Original: "Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation. " Modified to: "Subjects on apheresis should be on a stable frequency of the procedure at least 12 weeks prior to screening, with no planned change of frequency during the study participation *except as required by the protocol."*	Clarification that changes to the apheresis schedule are allowed during the study as described in Section 6.5.1.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria.
Inclusion Criterion #11, removal of requirement for participation in a long-term follow-up study as an inclusion criterion. Deleted Inclusion Criterion #11 ~~Willing to participate in a long-term follow-up study for up to 15 years after completion of this study.~~	The requirement for up to a 15-year follow-up after CTX310 infusion has already been built into the study design and in Section 8.1.1.5 and need not be an eligibility criterion. The subjects will be asked to participate in a separate LTFU study for up to 15 years post-infusion.	Section 1.1 Protocol Synopsis, Section 4.1 Inclusion Criteria
Exclusion Criterion #3 addition of neutrophil and lymphocyte cell count Original: Complete blood count: ~~White blood cell <2,500 cells/µL (2.5 × 10^9/L);~~ hemoglobin <11 g/dL (6.83 mmol/L) for males, <10 g/dL (6.21 mmol/L) for females; or platelet count <100,000/µL (100 ×10^9/L). Modified to: "Complete blood count: *neutrophils <1000 cells/µL (1.0 × 10^9/L); lymphocytes <500 cells/µL (0.5 × 10^9/L)*; hemoglobin <11 g/dL (6.83 mmol/L) for males, <10 g/dL (6.21 mmol/L) for females; or platelet count <100,000/µL (100 ×10^9/L)."	Instead of white blood cell count, specified neutrophil and lymphocyte count for exclusion of subjects with neutropenia and lymphocytopenia per se.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria

Change	Rationale	Affected Section(s)
Exclusion Criterion #5 addition of testing result requirements. Original: "Diagnosis of nephrotic syndrome" Modified to: "Diagnosis of nephrotic syndrome *or albuminuria >2+ on urine dipstick or albumin to creatinine ratio of >300 mg/g.*"	Addition of testing result requirements added for clarification.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Exclusion Criterion #7 modification of text Original: "History of alcohol or drug abuse." Modified to: "History of alcohol or *substance use disorder*."	Revision of language to accommodate diagnosis description as per DSM-5	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Exclusion Criterion #20 clarification; removal of selective serotonin reuptake inhibitor Original: "Current use of ~~selective serotonin reuptake inhibitors,~~ chronic systemic corticosteroid therapy, or anabolic agents." Modified to: "Current use of chronic systemic corticosteroid therapy, or anabolic agents."	Removal of selective serotonin reuptake inhibitors from the list of prohibited medications as their use will have minimal effect on assessment of lipid levels post-CTX310 administration as the subjects will continue SSRIs during the course of the study	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria,
Exclusion Criterion #21 clarification Original: "Current use of niacin-based supplements or nutraceuticals that may influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit." Modified to: "Current use of *nutraceuticals (e.g., niacin-based supplements or fish oil or red yeast rice) that significantly* influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit."	Addition of text to include both niacin-based or other nutraceuticals known to significantly influence lipid levels	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria Section 6.5.2 Prohibited Medications
Exclusion Criterion #23 clarification Original: "Positive serology for HIV-1 or HIV-2, hepatitis B virus (hepatitis B core antibody or NAT), or hepatitis C virus (NAT). Modified to: "Positive serology for HIV type 1 *or type 2,* hepatitis B virus (hepatitis B core antibody *or hepatitis B surface antigen or NAT*), or hepatitis C virus (*hepatitis C antibody testing* or NAT*)*."	Addition of options for testing to allow flexibility to clinical sites	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria

Change	Rationale	Affected Section(s)
Exclusion Criterion #25 clarification Original: "Any prior or current malignancy or myeloproliferative disorder or a significant immunodeficiency disorder." Modified to: "Any prior or current malignancy *except for the following that have been completely resected or removed: basal cell carcinoma, squamous cell carcinoma in situ, and carcinoma in situ of cervix or breast,* or myeloproliferative disorder or a significant immunodeficiency disorder."	Addition of text to account for the high prevalence of skin cancer in Australia and New Zealand and the curability following full resection	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Addition of new Exclusion Criterion #28 "Abnormal finding of myocardial ischemia on cardiac stress imaging (stress echocardiogram or radionuclide myocardial perfusion imaging [rMPI]) at screening. If results are inconclusive, findings should be discussed with the Safety Review Committee (SRC) prior to enrollment."	Addition of a new exclusion criterion for additional safety screening for ASCVD prior to infusion of CTX310	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Addition of new Exclusion Criterion #29 *Administration of vaccines 30 days before CTX310 infusion.*	Addition of a new exclusion criterion in alignment with the prohibition of vaccine administration 30 days before CTX310 infusion.	Section 1.1 Protocol Synopsis, Section 4.2 Exclusion Criteria
Study Procedures		
Section 1.2 Study Schema, new footnote 'c': "[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for rollover into a separate LTFU study for up to 15 years post-infusion."	Clarified the informed consent signature for the long-term follow-up study.	Section 1.2 Study Schema
Section 6.1 Administration of CTX310: addition of text regarding criteria for eligibility for administration of CTX310 on Day 1. Added text: "*Prior to administration of CTX310 on Day 1, investigator should confirm the ability of the subject to receive the infusion (see Table 1) by ensuring:* • *No significant change in clinical status since screening.* • *No new, clinically significant findings seen on physical exam, vital signs or ECG.* • *No AST, ALT, total bilirubin >2 × ULN and PT (INR) >1.5 × ULN.* *If the infusion is delayed for more than 30 days, the subject will be replaced if deemed necessary for dose escalation decisions*."	Addition of text to confirm eligibility	Section 6.1 Administration of CTX310

Change	Rationale	Affected Section(s)
Section 6.5.1 Allowed Medications and Procedures; added text regarding use of SSRIs and HRT *"Subjects previously prescribed selective serotonin reuptake inhibitors (SSRIs) or hormone replacement therapy (HRT) should remain on a stable dose from at least 30 days prior to screening through the end of the study."*	Addition of text to allow stable SSRI or HRT dosing.	Section 6.5.1 Allowed Medications and Procedures
Section 6.5.1 Allowed Medications and Procedures Original: "The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3, ~~icosapent ethyl,~~ evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study." Modified to: "The dose and regimen of TG- or LDL-C-lowering therapies (i.e., statins, ezetimibe, lomitapide, bile acid sequestrants, niacin, fibrates, omega-3 *(e.g., ethyl esters of EPA or DHA), bile acid sequestrants*, evinacumab, and/or PCSK9 monoclonal antibody or inclisiran, if applicable) are to remain constant from at least 30 days prior to screening through the end of the study.	Added examples of omega-3 supplements for clarity	Section 6.5.1 Allowed Medications and Procedures
Section 6.5.2 Prohibited Medications: deletion of SSRIs ~~• Selective serotonin reuptake inhibitors.~~ Addition of prohibition of vaccine administration and oral anticoagulants in the time period proximate to administration of study drug. *"All oral anticoagulants (e.g., warfarin, apixaban, rivaroxaban, or dabigatran) should not be administered 30 days before or 30 days after infusion of CTX310."* *"Vaccines should not be administered 30 days before or 30 days after infusion of CTX310."*	SSRIs are allowed at a constant dose – see Section 6.5.1. Vaccines and oral anticoagulants should not be administered 30 days before or after infusion of CTX310.	Section 6.5.2 Prohibited Medications
Section 6.5.2 Prohibited Medications: Rearranged the statement and added fish oil or red yeast rice as examples of nutraceuticals. ~~Niacin or~~ Nutraceuticals *(e.g., niacin*-based supplements or ~~nutraceuticals~~ *fish oil or red yeast rice)* that ~~may~~ *significantly* influence lipid levels at a dose/amount that has not been stable for >30 days prior to the screening visit.	Addition of examples of nutraceuticals that may alter lipid levels.	Section 6.5.2 Prohibited Medications
Section 6.6 Lifestyle Considerations Addition of prohibition of any tissue/blood donation for the duration of the study. *Subjects who receive CTX310 must not donate eggs, sperm, blood, or organs for the duration of this study.*	Addition of prohibition of any tissue/blood donation for the duration of the study.	Section 6.6 Lifestyle Considerations

Change	Rationale	Affected Section(s)
Section 8.1.1.1 Screening and Enrollment; added "Cardiac Stress Imaging" Original: "Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, echocardiogram, and infectious disease markers (if these procedures were performed within 60 days prior to infusion)." Modified to: "Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-*PDFF*/ultrasound of liver, *cardiac stress imaging,* echocardiogram, and infectious disease markers (if these procedures were performed within 60 days prior to infusion)."	Added stress imaging to the list of screening that do not need to be repeated upon rescreening within 60 days prior to infusion	Section 8.1.1.1
Section 8.1.1.5 Long-term Follow-up; clarified text Original: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310, and either discontinue or complete this study, will be asked to participate in a separate LTFU study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events." Modified to: "To comply with local regulatory requirements/guidance for subjects administered a gene therapy, all subjects who receive an infusion of CTX310, and either discontinue *prior to 12 months* or complete this study, will be asked to participate in a separate LTFU study for up to 15 years post-infusion to assess long-term safety, durability, and effect on cardiovascular events. "	Clarified the text describing LTFU to include those who discontinue prior to12 months	Section 8.1.1.5 Long-term Follow-up
Section 8.2.1 Informed consent, new text added: "*All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to rollover into a separate LTFU study for up to 15 years post-infusion (see Section 8.1.1.5 and Table 1*)."	Clarified the informed consent signature for the LTFU study.	Section 8.2.1 Informed Consent
Section 8.2.3 Physical Examination, Height, and Weight Original: "Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, Day 30, and EOS visits, and the results documented." Has been modified to: "Complete physical examination, including general appearance, skin, neck, head, eyes, ears, nose, throat, heart, lungs, abdomen, lymph nodes, extremities, and nervous system, will be performed at the screening, *Day 1*, Day 30, and EOS visits, and the results documented."	Added a full physical exam on Day 1	Section 8.2.3 Physical Examination, Height and Weight

Change	Rationale	Affected Section(s)
New Section 8.2.6 Cardiac Stress Imaging; added new text *A stress echocardiogram or rMPI (single-photon emission computed tomography [SPECT] or positron emission tomography [PET]) will be performed before CTX310 administration at screening as described in the schedule of assessments (Table 1). Exercise or a pharmacologic agent may be used to induce stress conditions. Standard local regulations will be used to image acquisition and interpretation. If the results of the stress imaging are not clearly interpretable for the subjects, the sponsor may approach the SRC for a decision regarding eligibility.*	Additional safety screening for ASCVD prior to infusion of CTX310	Section 8.2.6 Cardiac Stress Imaging
Section 8.2.8 Electrocardiogram; Addition of requirement for Day 1 ECG prior to CTX310 infusion. Original: "Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion." Modified to: "Twelve (12)-lead ECGs will be obtained as described in the schedule of assessments (Table 1). *Day 1 ECG will be collected prior to pre-infusion prophylaxis regimen.* QTc and QRS intervals will be determined from ECGs. Additional ECGs may be obtained at the investigator's discretion."	Clarification that Day 1 ECG will be collected prior to the pre-infusion prophylaxis regimen.	Section 8.2.8 Electro-cardiogram
Section 8.2.9 Laboratory Tests; clarification of assessments Serum chemistry: Original: "ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein" Modified to: ""ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN/*urea*, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein" Urinalysis: Original: "Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal)" Modified to: "Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal), *albuminuria (dipstick) or urine ACR"*	Clarification of laboratory testing to be performed at the local level.	Section 8.2.9 Laboratory Tests, Table 4 Local Laboratory Testing, Table 5 Central Testing

Change	Rationale	Affected Section(s)
Coagulation Original: "PT, PTT, fibrinogen" Modified to: "PT *(INR),* PTT/*aPTT*, fibrinogen" CBC with differential: Original: "Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count" Modified to: "Hematocrit, hemoglobin, red blood cell count, white blood cell count, neutrophils, lymphocytes, monocytes, basophils, eosinophils, platelet count, absolute neutrophil count, *absolute lymphocyte count. Note: The white blood cell count differential may be reported as absolute counts or as percentages, with the exception that absolute neutrophil and lymphocyte counts are required for eligibility assessment* Viral serology Original: "HIV-1, HIV-2, HCV antibody ~~and RNA~~, HBV surface antigen, HBV surface antibody, HBV core antibody" Modified to: "HIV-1, HIV-2, HCV antibody *or NAT*, HBV surface antigen, HBV surface antibody, HBV core antibody *or NAT"* Table 5 Central Testing Footnote 1 added: "*Collection of a sample for genetic testing should not be repeated during rescreening, if applicable."*		
Section 8.2.10 deletion of "~~enrolled in the study~~" for consistency with Table 1 footnote and "~~A negative serum pregnancy test, performed as per local standards, is required within 72 hours before CTX310 infusion~~."	Pregnancy test at Day 1 is not required as it will be performed at screening, as per local standard, for this trial that will enroll women who meet the criteria of being non-childbearing. Redundant text deleted from Table 1 Footnote 10.	Section 8.2.10 Pregnancy Testing

Change	Rationale	Affected Section(s)
Safety, Adverse Events, and Study Oversight		
Clarification of follow-up for AEs. Added text: "*AEs will be followed through to event resolution or stability or death*."	Clarification of language.	Section 9 Adverse Events
Section 9.11.1 Safety Review Committee addition of text regarding additional consultation The SRC may be consulted on other aspects of the study conduct, as applicable.	Addition of text for consultation with SRC regarding eligibility when data are not clearly interpretable.	Section 1.1 Protocol Synopsis; Section 9.11.1 Safety Review Committee
Stopping Rule		
Section 10.1 clarification of stopping rule 3. Original: "3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5." Will be modified to: "3. ALT or AST >3 × ULN (or the greater of 2 × baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or INR >1.5 *persisting for >2 weeks*".	Addition of a time limit on the allowable duration of elevated ALT, AST, or total bilirubin to avoid unnecessary stoppages in the study.	Section 10.1 Stopping Rules for the Study
General Change		
All relevant laboratory measurement values will also be expressed in the International System of Units (SI) units.	For operational consistency.	Relevant sections throughout the protocol including Inclusion/ Exclusion criteria, as applicable.
All instances of "participant" were changed to "subject" and all instances of "cohort" were changed to "dose level". [redacted] was a typo and was corrected to [redacted] throughout. Minor editorial and formatting changes throughout as applicable. Section numbering was updated based on additions and deletions. Updated protocol version and date and Medical Monitor signature.		

ACR: albumin to creatinine ratio; AE: adverse event; AESI: adverse event of special interest; ALT: alanine aminotransferase; ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; aPTT: activated partial thromboplastin time; ASCVD: atherosclerotic cardiovascular disease: AST: aspartate aminotransferase; BUN: blood urea nitrogen; CVD: cardiovascular disease; D or d: day; DHA: docosahexaenoic acid; DL: Dose Level; DLT: dose limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; EL: endothelial lipase; EOS: end of study; EPA: eicosapentaenoic acid; FFA: free fatty acids; HbA1c: glycosylated hemoglobin; HBV: hepatitis B virus; HCV: hepatitis C virus; HDL-C: high-density lipoprotein cholesterol; HIV: human immunodeficiency virus; HRT: hormone replacement therapy; INR: international normalized ratio; LDL-C: low-density lipoprotein cholesterol; LTFU: long-term follow-up; LVEF: left ventricular ejection fraction; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI: magnetic resonance imaging; NAT: nucleic acid testing; OBD: optimum biological dose; PCSK9: proprotein convertase subtilisin/kexin type 9; PDFF: protein density fat fraction;

PET: positron emission tomography; PK: pharmacokinetic; PT: prothrombin time; PTT: partial thromboplastin time; rMPI: radionuclide myocardial perfusion imaging; RNA: ribonucleic acid; RP2D: recommended Phase 2 dose; SGOT: serum glutamic oxaloacetic transaminase; SGPT: serum glutamic pyruvic transaminase; SI: International System of Units; SPECT: single-photon emission computed tomography; SRC: Safety Review Committee; SSRI: selective serotonin reuptake inhibitor; TEAE; treatment-emergent adverse event; TG; triglyceride(s); ULN: upper limit of normal; VLDL: very low-density lipoprotein.

14.4.6. Summary of Changes to Global Protocol V2.0, Amendment 1

Protocol CRSP-CVD-400 Version 2.0 (Amendment 1), dated 05 May 2023, was internally approved per the sponsor's standard operating procedure. However, Version 1.0 dated 27 January 2023 was not submitted to a health authority, Institutional Review Board, or Ethics Committee and was revised to Version 2.0, dated 05 May 2023, to reflect changes to the study conduct based on the sponsor's decision.

14.4.7. Protocol History

Document/Version	Date	Global/Country/Site Specific
Original Protocol/Version 1.0	27 January 2023	Global
Protocol Amendment 1/Version 2.0	05 May 2023	Global
Protocol Amendment 2/Version 3.0	08 December 2023	Global
Protocol Amendment 3/Version 4.0	12 January 2024	Global
Protocol Amendment 4/Version 5.0	03 June 2024	Global
Protocol Amendment 4/Version 5.1	17 January 2025	Country Specific
Protocol Amendment 5/Version 6.0	08 April 2025	Global
Protocol Amendment 5/Version 6.1	18 April 2025	United Kingdom (UK) Specific

15. APPENDIX: GLOSSARY OF TERMS

List of Abbreviations

Abbreviation	Term
ACC	American College of Cardiology
ADA	anti-drug antibody
AE	adverse event
AESI	adverse event of special interest
AHA	American Heart Association
ALT	alanine aminotransferase
ANGPTL3	angiopoietin-like 3
AP	alkaline phosphatase
ApoB	apolipoprotein B
ASCVD	atherosclerotic cardiovascular disease
AST	aspartate aminotransferase
BMI	body mass index
Cas9	CRISPR-associated protein 9
CCS	Canadian Cardiovascular Society
CNS	central nervous system
CRF	case report form
CRISPR	clustered regularly interspaced short palindromic repeats
crRNA	crispr RNA
CTCAE	Common Terminology Criteria for Adverse Events
CVD	cardiovascular disease
DHA	docosahexaenoic acid
DL	Dose Level
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DP	drug product
DS	drug substance
DSB	double-stranded break
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
ECG	electrocardiogram
eCRF	electronic case report form
EL	endothelial lipase

Abbreviation	Term
eLBW	estimated lean body weight
EOS	end of study
EPA	eicosapentaenoic acid
FAS	full analysis set
FCS	familial chylomicronemia syndrome
FDA	Food and Drug Administration
FFA	free fatty acids
FIH	first-in-human
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GOF	gain-of-function
GP1HBP1	glycosylphosphatidylinositol anchored high-density lipoprotein binding protein 1
HDL-C	high-density lipoprotein cholesterol
HDR	homology directed repair
HeFH	heterozygous familial hypercholesterolemia
HIV	human immunodeficiency virus
HoFH	homozygous familial hypercholesterolemia
HRT	hormone replacement therapy
ICF	informed consent form
ICH	International Conference on Harmonisation
IDL	intermediate-density lipoprotein
IgM	immunoglobulin M
indel	insertion or deletion
INR	international normalized ratio
IRB	Institutional Review Board
IRR	infusion-related reaction
IV	intravenous
kPa	kilopascals
LDL-C	low-density lipoprotein cholesterol
LDLR	low-density lipoprotein receptor gene
LFT	liver function test
LNP	lipid nanoparticle
LOF	loss-of-function
Lp(a)	lipoprotein(a)
LPL	lipoprotein lipase

Abbreviation	Term
LTFU	long-term follow-up
LVEF	left ventricular ejection fraction
MHRA	Medicines & Healthcare Products Regulatory Agency
MRE	magnetic resonance elastography
MRI-PDFF	magnetic resonance imaging-protein density fat fraction
mRNA	messenger ribonucleic acid
MTD	maximum tolerated dose
NAT	nucleic acid testing
NHEJ	nonhomologous end joining
NHP	non-human primate
NOAEL	no-observed-adverse-effect level
OBD	optimum biological dose
PAM	protospacer adjacent motif
PCSK9	proprotein convertase subtilisin/kexin type 9
PD	pharmacodynamic(s)
PDFF	protein density fat fraction
PK	pharmacokinetic(s)
PT	prothrombin time
RNA	ribonucleic acid
RP2D	recommended Phase 2 dose
SAE	serious adverse event
sgRNA	single-guide RNA
SpCas9	*Streptococcus pyogenes* CRISPR-associated protein 9
SRC	Safety Review Committee
SSRI	selective serotonin reuptake inhibitor
TEAE	treatment-emergent adverse event
TG	triglyceride(s)
tracrRNA	trans-activating crispr RNA
UK	United Kingdom
ULN	upper limit of normal
VLDL	very low-density lipoprotein

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Signature Page

Workflow Step: Approval	Name: [redacted] Signature Capacity: Clinical Development Date: 18-Apr-2025 18:18:00 GMT+0000

E-signatures are binding of traditional handwritten signatures. E-record data is stored in CRISPR's validated DMS and compliant with regional requirements.

CLINICAL STUDY PROTOCOL
CRSP-CVD-400

A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias

Study Drug: CTX310
Study Phase: 1

SUMMARY OF CHANGES

CRISPR Therapeutics AG
Baarerstrasse 14
CH 6300 Zug, Switzerland

TABLE OF CONTENTS

Protocol History

Document/Version	Date	Global/Country/Site Specific
Original Protocol/Version 1.0	27 January 2023	Global
Protocol Amendment 1/Version 2.0	05 May 2023	Global
Protocol Amendment 2/Version 3.0	08 December 2023	Global
Protocol Amendment 3/Version 4.0	12 January 2024	Global
Protocol Amendment 4/Version 5.0	03 June 2024	Global
Protocol Amendment 4/Version 5.1	17 January 2025	Country Specific
Protocol Amendment 5/Version 6.0	08 April 2025	Global
Protocol Amendment 5/Version 6.1	18 April 2025	United Kingdom (UK) Specific

Summary of Changes from Version 1.0 to Version 2.0, Amendment 1

The previous version of this protocol (Version 1.0, 27 January 2023 was amended to create the current version (Version 2.0, 05 May 2023). Key changes made in the current version of the protocol are summarized below.

- Familial chylomicronemia syndrome (FCS) was removed from the population.
- Dosing of CTX310 was updated to be based on estimate lean body weight (eLBW).
- An option for future protocol amendment to add a dose expansion cohort was added.
- Dose level (DL) 4 has been revised from 1 mg/kg to 0.8 mg/kg eLBW.
- Dose escalation rules have been modified to include optional dose de-escalation at DL3 and DL4
- Duration of hospitalization following infusion of CTX310 was decreased from 72 hours to a minimum of 24 hours and safety monitoring visits have been added on Day 3 and Day 4 with the requirement that the participant stay within 1 hour of the infusion site.
- The requirement for abstinence from alcohol for the duration of the study has been eliminated. Guidance regarding allowable alcohol consumption during the study has been added.
- Clear instruction for vital sign assessment frequency was provided for Day1 until discharge from the hospital.
- Clarified instruction regarding timing of Day 1 laboratory values and repeating serum chemistry to define adverse event of special interest and stopping rules.
- Clarified timing of samples for genetic testing.
- Clarified all samples for lipid panels should be performed in a fasting state.
- Removed requirement for blood samples for storage to be stored in PAXgene tubes.
- Lomitapide has been added as a standard of care for hyperlipidemia.
- The requirement for 365-day hiatus from use of antisense oligonucleotide molecule has been eliminated.

Summary of Changes from Version 2.0 to Version 3.0, Amendment 2

The previous version of this protocol (Version 2.0, 05 May 2023) was amended to create the current version (Version 3.0, 08 December 2023). This amendment is not likely to have a substantial impact on safety or rights of the study subjects, or on the reliability and robustness of the data generated in the clinical trial.

The primary reason for this amendment is to incorporate the Ethics Committee and regulatory feedback and clarifications of procedures, including:

- Confining Primary Objective to dose-limiting toxicity (DLTs)
- Adding new Secondary Safety Objective and Endpoint
- Clarification of description of the study population
- Clarification of Inclusion Criterion
- Removal of Inclusion Criterion #11
- Addition of new Exclusion Criteria and clarification of Exclusion Criterion
- Adding criteria to be met prior to administration of CTX310 on Day 1 for the investigator to confirm the ability of the subject to receive the infusion.
- Updated Section 6.5.2, Prohibited Medications, to align with the exclusion criteria
- Addition of prohibition of tissue/ blood donation to Section 6.6, Lifestyle Considerations
- Adding a new Section 8.2.6 for Cardiac Stress Imaging at screening
- Clarification of Stopping Rule 3 in Section 10.1.

Summary of Changes from Version 3.0 to Version 4.0, Amendment 3

The previous version of this protocol (Version 3.0, 08 December 2023) was amended to create the current version (Version 4.0, 12 January 2024). This amendment is not likely to have a substantial impact on safety or rights of the study subjects, or on the reliability and robustness of the data generated in the clinical trial.

The key changes to global protocol Amendment 3 (V4.0) are:

- to remove the cardiac stress imaging during screening for exclusion of subjects with abnormal myocardial ischemia. It was added to Amendment 2 (Version 3.0) of the protocol by the sponsor to enable assessment of clinically relevant atherosclerotic cardiovascular disease (ASCVD) prior to administration. However, based on feedback from the study investigators, it may result in exclusion of the intended study population, thus impacting enrollment. The protocol already includes echocardiogram and 12-lead electrocardiogram (ECG) assessments during screening for decision regarding eligibility.
- test the elevation of cardiac biomarker (troponin I or T) before the administration of CTX310.

Summary of Changes from Version 4.0 to Version 5.0, Amendment 4

The previous version of this protocol (Version 4.0, 12 January 2024) was amended to create the current version (Version 5.0, 03 June 2024). This amendment is not likely to have a substantial impact on safety or rights of the study subjects, or on the reliability and robustness of the data generated in the clinical trial.

The key changes to global protocol Amendment 4 (V5.0) are:

- Revision of Inclusion Criterion #1 to raise the upper age limit from ≤70 to ≤75 years to be more inclusive of the intended study population.
- Revision of Inclusion Criterion #3 to lower the limit of triglycerides to >150 mg/dL (1.7 mmol/L) to be more inclusive of the intended study population.
- Revision of Exclusion Criterion #1 to exclude subjects with familial chylomicronemia syndrome with <5% lipoprotein lipase (LPL) activity.
- Addition of new Exclusion Criterion #11 regarding severe aortic stenosis.
- Deletion of Exclusion Criterion #18 regarding use of monoclonal antibodies.
- Revision of Exclusion Criterion #25 to exclude subjects with prior malignancy within 5 years for inclusion of subjects who are in remission.
- Addition of measurement of complete blood count on Days 1, 3, 7 and 14, to support monitoring of adverse events to Table 1 Schedule of Assessments.
- Addition of option for second dose for eligible subjects once the recommended Phase 2 dose (RP2D) has been selected.
- Addition of the following DLT criteria to capture hepatotoxicity, hepatocyte damage or synthetic liver dysfunction:
  - Any CTCAE grade ≥3 elevations in ALT and AST that persist for >14 days and is assessed by the investigator as related to investigational product.
  - Any CTCAE grade ≥3 elevations in serum bilirubin or prothrombin time (International Normalized Ratio [INR]) and is assessed by the investigator as related to investigational product.
  - Any CTCAE grade ≥3 decrease in platelet count and is assessed by the investigator as related to investigational product.
- To improve alignment with expected hepatotoxicities, specified that the treatment of the next subject will be paused if any of the first 4 stopping rule criteria are met in ≥2 subjects at a dose level. Specified the duration of persistence of ALT or AST elevations ≥8×ULN.
  - ≥2 subjects at a DL with ALT or AST >8 × ULN, which is confirmed and persists for >14 days.
  - ≥2 subjects at a DL with ALT or AST >5 × ULN, which is confirmed and persists for >30 days.

- ≥2 subjects at a DL with ALT or AST >3× ULN (or the greater of 2× baseline value or 3 × ULN if the baseline value was >ULN), which is confirmed, with concomitant increase in total bilirubin >2 × ULN or international normalized ratio >1.5 × ULN persisting for >14 days.

Summary of Changes from Version 5.0 to Version 6.0, Amendment 5

The previous version of this protocol (Version 5.0, 03 June 2024) was amended to create the current version (Version 6.0, 18 April 2025).

This is a substantial amendment to the protocol. The primary reason for this amendment is:

- Replacement of the confirmatory cohort from Phase 1a with Phase 1b disease-specific cohort expansion using a flat dose within a recommended range approved by the Safety Review Committee (SRC) following evaluation of the totality of pharmacokinetic (PK)/pharmacodynamic (PD), key lipid and safety data from dose escalation based on estimated lean body weight in mg/kg of CTX310.
- Addition of 4 disease specific treatment cohorts in a Phase 1b cohort expansion: severe hypertriglyceridemia, homozygous familial hypercholesterolemia, heterozygous familial hypercholesterolemia and refractory mixed hyperlipidemias.
- Addition of a disease-specific exploratory objective/endpoint.
- Addition of disease specific inclusion/exclusion criteria.
- Update of pre-infusion prophylaxis regimen.

Country-specific Amendments

Summary of Changes from Version 5.0 to UK Version 5.1, Amendment 5

This is a substantial country specific amendment (Version 5.1, 17 January 2025) to the global protocol CRSP-CVD-400 Version 5.0 (03 June 2024), for the United Kingdom (UK), in response to the request received from Medicines & Healthcare Products Regulatory Agency (MHRA).

Summary of Key Changes to the Country Specific Protocol Version 5.1, Amendment 4 (UK)

The key changes based on MHRA request are:

- Revision of Inclusion Criteria #3 and #4 and the Investigational Plan Section **Error! Reference source not found.** to limit the inclusion of participants eligible for CTX310 to the following criteria to provide an appropriate benefit risk balance:
  a. Subjects with established atherosclerotic cardiovascular disease and not at LDL-C target on maximum tolerated dose of hydroxymethylglutaryl CoA reductase inhibitors (statins)
  b. Subjects with established atherosclerotic cardiovascular disease and who are statin-intolerant, or for whom a statin is contraindicated
  c. Subjects with Homozygous familial hypercholesterolemia (HoFH)
  d. Subjects with Heterozygous familial hypercholesterolemia (HeFH)
- Revised exclusion criterion #1 to exclude subjects with chylomicronemia syndromes (e.g., familial chylomicronemia syndrome [FCS]).
- The exclusion criterion #2a "total bilirubin value >2 × ULN" reduced to > 1.5 × ULN as a level of > 2 × ULN may indicate a clinically significant level of liver injury already present.
- Revised the exclusion criterion #3 that relates to neutrophil count to $<1.5 \times 10^9$/L, as a level below that indicates a substantial increase in the risk of infection, and the benefit risk balance in this first-in-human trial of experimental therapy indicates that a lower level is inappropriate.
- Revised the threshold of HbA1c to ≤8% in exclusion criterion #6 as a higher level may reflect metabolic instability which is inappropriate for a first-in-human study for gene therapy.
- Revised the exclusion criterion #9 that addressed uncontrolled or untreated thyroid disease to <0.5 and >5.0 mIU/L, to align with the usual window of "controlled" thyroid function within 0.5 to 5.0 mIU/L.
- Reordered and clarified the first criterion in the definition of Dose Limiting Toxicity (DLT) to be the last in the list of DLT criteria and to state "Any other CTCAE grade ≥3 AE, other than those listed in bullets above, that is assessed by the investigator as related to investigational product". This clarification was made to eliminate any redundancy with other definitions that propose to qualify an event as a DLT criterion based on a laboratory abnormality. Deleted the criterion, "Any CTCAE grade ≥3

decrease in platelet count that is assessed by the investigator as related to investigational product", as this is redundant with the clarified and reordered bullet#.

- Modified the schedule of cardiac biomarker test in the Schedule of Assessments to also repeat at Days 7, 30, and 90.
- Revision of Protocol Section 9.11.1 (Safety Review Committee [SRC]) to add the following final paragraph

  "Any revisions to the dose limiting toxicity (DLT) definitions or criteria, dosing schema can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented."
- Revision of Protocol Section 9.11.2 (Independent Data Safety Monitoring Board [DSMB]) to add the following paragraph

  "Any DSMB recommended revisions to the protocol can only be implemented after approval by the regulatory authority of an application for a protocol amendment in which the data and rationale are presented."
- Protocol Section 10.1 modified to indicate that no changes can be implemented until the application is approved by the regulatory authority.
- Protocol Section 10.1 modified to state that if any stopping rule is met, the study can only be re-started after an amendment application that presents the data and the rationale to re-start is approved by the regulatory authority

Summary of Changes from UK Version 5.1 to UK Version 6.1, Amendment 5 (UK)

This is a substantial amendment to the protocol. The primary reason for this amendment is:

- Replacement of the confirmatory cohort from Phase 1a with Phase 1b disease-specific cohort expansion using a flat dose within a recommended range approved by the Safety Review Committee (SRC) following evaluation of the totality of pharmacokinetic (PK)/pharmacodynamic (PD) and safety data from dose escalation based on estimated lean body weight in mg/kg of CTX310.
- Further clarified the study population as follows (in alignment with MHRA feedback incorporated in the prior protocol version [V5.1_Amendment 4] submitted):
  - Severe HTG with history of ASCVD (secondary prevention only)
  - HoFH (primary or secondary prevention of ASCVD)
  - HeFH (primary or secondary prevention of ASCVD)
  - Mixed hyperlipidemias with history of ASCVD (secondary prevention only)
- Addition of a disease-specific exploratory objective/endpoint
- Addition of disease specific inclusion/exclusion criteria
- Update of pre-infusion prophylaxis regimen

Statistical Analysis Plan

A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (*ANGPTL3)* Gene in Subjects With Refractory Dyslipidemias

Clinical Study Protocol CRSP-CVD-400

Version History of Implemented Plans

Version	Date
1.0	27AUG2025

SIGNATURE PAGE

I have prepared the SAP as a subject matter expert in Biostatistics and I approve the contents:

[redacted] 27-Aug-2025

[redacted] Date

I have reviewed the SAP as a subject matter expert in Clinical Development and I approve the contents:

[redacted] 27-Aug-2025

[redacted] Date

Table of Contents

List of Abbreviations and Definitions of Terms

AE	Adverse Event
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
Cas9	CRISPR-associated protein 9
CI	Confidence Interval
CNS	Central Nervous System
CRF	Case Report Form
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DES	DLT Evaluable Set
DLT	Dose-limiting Toxicity
DSMB	Data Safety Monitoring Board
eLBW	Estimated Lean Body Weight
ECG	Electrocardiogram
FAS	Full Analysis Set
HDL	High Density Lipoprotein
HTG	Hypertriglyceridemia
ICF	Informed Consent Form
LDL	Low Density Lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum Tolerated Dose
PK	Pharmacokinetic(s)
PT	Preferred Term
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Safety Analysis Set
SD	Standard Deviation
SOC	System Organ Class

SRC	Safety Review Committee
TEAE	Treatment-Emergent Adverse Event

1. INTRODUCTION

This statistical analysis plan (SAP) describes the analysis for study, "A Phase 1 Open-label, Multicenter, First-in-human, Ascending Single-dose Study Evaluating the Safety and Tolerability of a Lipid Nanoparticle Formulation of CRISPR–Guide RNA–Cas9 Nuclease (CTX310) for In Vivo Editing of the Angiopoietin-like 3 (ANGPTL3) Gene in Subjects With Refractory Dyslipidemias" version 6.0 (amendment 5) which was issued on 18 April 2025. This SAP provides a comprehensive and detailed description of the strategy, rationale, and statistical techniques to evaluate the necessary safety and efficacy endpoints. The purpose of this SAP is to ensure the credibility of the study findings by prespecifying the statistical approaches to the analysis of study data prior to database lock. For pharmacokinetics/pharmacodynamics (PK/PD) and exploratory endpoints, the analyses included in this SAP are of descriptive nature. Additional analyses on PK/PD and exploratory endpoint data other than the ones specified in this SAP may be performed, and will be described in a separate analysis plan if necessary.

2. STUDY OBJECTIVES AND ENDPOINTS

Primary Objectives	Primary Endpoints
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of DLTs and frequency of AEs
Secondary Objectives	**Secondary Endpoints**
• To assess the preliminary efficacy of CTX310	• Percentage change in TG, ApoB, non–HDL-C, LDL-C, and HDL-C concentrations over time compared to baseline
• To further characterize the safety of CTX310	• Frequency and severity of AEs, including TEAEs and AESIs, clinically significant laboratory abnormalities, and clinically significant abnormal vital signs
• To assess the PK of CTX310	• Plasma levels of LNP ([redacted] and [redacted]) • Plasma level of Cas9 protein
• To assess the PD of CTX310	• Percentage change in ANGPTL3 concentration over time compared to baseline
Exploratory Objectives	**Exploratory Endpoints**
• To identify changes associated with CTX310 that may indicate or predict clinical response, immunogenicity, safety, or PD activity	• Percentage change in FFA levels over time compared to baseline. • Change in fatty liver disease. • Immunogenicity of CTX310 (samples will be stored and evaluated for ADA to LNP and Cas9, if required). • For Phase 1b only: Change from baseline in number of acute pancreatitis events through 12 months in subjects with severe HTG • Percentage change in remnant cholesterol concentration over time compared to baseline

ADA: anti-drug antibody; AE: adverse event; AESI: adverse event of special interest; [redacted]

ANGPTL3: angiopoietin-like 3; ApoB: apolipoprotein B; Cas9: CRISPR-associated protein 9; DLT: dose-limiting toxicity; FFA: free fatty acid; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; LNP: lipid nanoparticles; Lp(a): lipoprotein(a); PD: pharmacodynamic(s); PEG: polyethylene glycol; PK: pharmacokinetics; TEAE: treatment-emergent adverse event; TG: triglycerides.

3. INVESTIGATIONAL PLAN

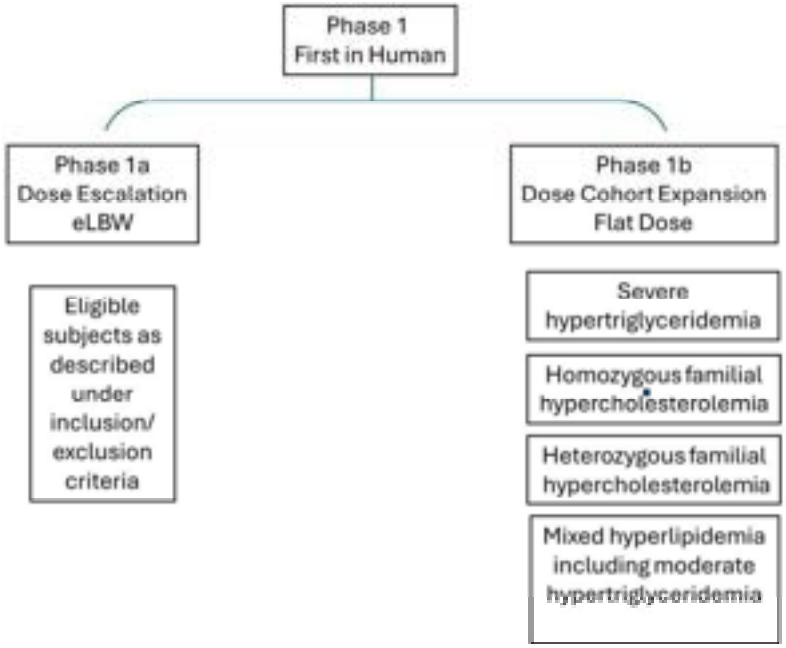
3.1 Overall Study Design

3.1.1. Dose escalation

Study CRSP-CVD-400 is a single-arm, open-label, multicenter, ascending single-dose Phase 1 study evaluating the safety, preliminary efficacy and pharmacokinetics/pharmacodynamics (PK/PD) of CTX310 in adult participants with refractory dyslipidemia. CTX310 is a lipid nanoparticle (LNP) formulation of CRISPR-Cas9 components for *in vivo* gene editing of the target gene *ANGPTL3.*

The study is divided into 2 parts (Figure 1): Phase 1a dose escalation followed by Phase 1b disease-specific cohort expansion. Phase 1a will delineate a flat dose (FD) for Phase 1b or anticipated recommended Phase 2 dose (RP2D). The FD will be a dose associated with optimal biological efficacy, as determined by the intended decrease in ANGPTL3 and key lipid levels with minimum toxicity. Phase1b will confirm the RP2D.

Figure 1: Study Design



The Phase 1a dose escalation part of this study will include subjects with the following monogenic or polygenic refractory dyslipidemias, with or without atherosclerotic cardiovascular disease (ASCVD), that encompass hypertriglyceridemia (HTG) and/or hypercholesterolemia syndromes:

- Multifactorial chylomicronemia syndrome (MCS).
- Homozygous familial hypercholesterolemia (HoFH).
- Heterozygous familial hypercholesterolemia (HeFH)
- Familial chylomicronemia syndrome (FCS) (with ≥5% lipoprotein lipase [LPL] activity).
- Other HTG/hypercholesterolemia syndromes of undetermined etiologies.

The Phase 1b disease-specific cohort expansion will include eligible subjects with the following monogenic or polygenic refractory dyslipidemias, with or without ASCVD that encompass HTG and/or familial hypercholesterolemia syndromes in the following disease-specific cohorts:

• Severe HTG (>500 mg/dL triglycerides)

• HoFH

• HeFH

• Mixed hyperlipidemias (including moderate HTG)

The dose levels of CTX310 to be tested in Phase 1a are listed in Table 1.

Table 1: Dose Escalation of CTX310.

Dose Level	Planned Dose (mg/kg eLBW) [1]
1	0.1
2	0.3
3	0.6
4	0.8 [2]
5	1.0 [3]
6	1.2 [4]

DL: Dose Level; eLBW: estimated lean body weight; RNA: ribonucleic acid; SRC: Safety Review Committee.
* Following review of clinical data by the sponsor and SRC, additional intermediate DLs, besides the prespecified ones, may be added during the course of dose escalation to support the identification of a safe and effective dose.
1 Dose of CTX310 is referred to by amount of total RNA administered, which is the total amount of guide RNA + messenger RNA per kg of eLBW.
2 Following review of clinical data by the sponsor and SRC at DL4, a de-escalation to a dose of 0.7 mg/kg eLBW may be explored.
3 Following review of clinical data by the sponsor and SRC at DL5, a de-escalation to a dose of 0.9 mg/kg eLBW may be explored.
4 Following review of clinical data by the sponsor and SRC at DL6, a de-escalation to a dose of 1.1 mg/kg of eLBW may be explored.

Dose escalation will be performed using a standard 3+3 design, according to the following rules:

- If 0 of 3 participants experience a DLT, escalate to the next dose level.
- If 1 of 3 participants experiences a DLT, expand the current dose level to 6 participants.
  - If 1 of 6 participants experiences a DLT, escalate to the next dose level.
  - If ≥2 of 6 participants experience a DLT in DLs 2, 3, or 4, de-escalate to previous dose level, or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- If ≥2 of 3 participants experience a DLT in DLs 2, 3, or 4, or any optional de-escalation dose level as specified in the table above, de-escalate to previous dose level or declare previous dose level the MTD if 6 participants are already tested at the previous dose level.
- No dose escalation is planned beyond the highest dose level listed for the study.

Following completion of eLBW-based dose escalation (Table 1), analysis of PK/PD and safety data, and approval by the SRC, a flat dose (FD) of CTX310 may be evaluated in a Phase 1b cohort expansion in 4 disease-specific cohorts.

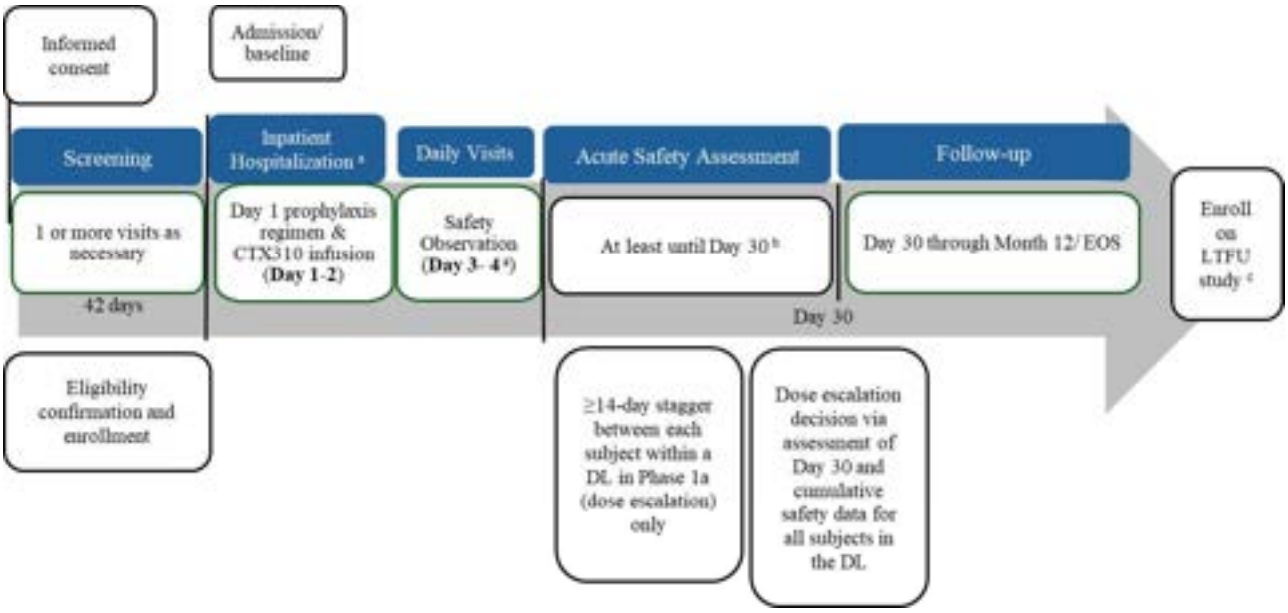
The SRC will endorse the RP2D based on the review of the totality of data including clinical activity and safety of the FD for disease-specific groups. Once the RP2D is determined, subjects who may have received a sub-efficacious dose of CTX310 may be eligible for a second dose at the RP2D based on predefined criteria provided in a future protocol amendment or separate study protocol.

In both Phase 1a and 1b, after CTX310 infusion, participants will be followed for 12 months with assessments for AEs, effects on lipid profile and *ANGPTL3* expression, regular laboratory evaluations and physical examinations. After completion of this study, all participants will be asked to participate in a separate long-term follow-up study for up to 15 years post-infusion.

Each participant will undergo the following stages (Figure 2) in the study:

1. Screening: from signing informed consent to eligibility confirmation and enrollment, up to 6 weeks.
2. Infusion of CTX310 (Day 1) and acute safety evaluation period (30 days post-infusion).
3. Follow-up: All participants will be monitored for safety, tolerability, PK, and PD effects for an additional 11 months after the acute safety evaluation period in the clinical study.
4. Long-term follow-up: Roll over to a separate long-term follow-up study for up to 15 years post-infusion.

Figure 2: Study Schema.



DL: dose level; EOS: end of study; LTFU: long-term follow-up.

[a] Inpatient hospitalization for CTX310 infusion on Day 1. Hospital discharge on Day 2 after the completion of safety evaluation and laboratory tests and a minimum of 24 hours after the completion of the CTX310 infusion. Daily safety visits on Day 3 and 4. Inpatient hospitalization may be extended or following hospital discharge the subject may be readmitted or additional daily safety visits beyond Day 4 may be required at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice.

[b] For each DL during Phase 1a dose escalation, there will be a safety monitoring period of ≥14 days between the treatment of each subject and any subsequent subjects within the DL, or until the subject is clinically stable and all laboratory values (including liver function tests) have returned to <2 × baseline or to normal levels, whichever is later. No stagger is required in Phase 1b.

[c] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to sign an informed consent for roll over into a separate LTFU study for up to 15 years post-infusion.

3.1.2. Study Oversight

A study Safety Review Committee (SRC) consisting of investigators and sponsor representatives will review all available safety data when the DLT observation period ends for the last participant enrolled in each cohort and will be responsible for making decisions regarding dose escalation or de-escalation. The SRC will continue to meet regularly during the dose escalation phase to discuss toxicity management algorithms and to review individual participant cases.

An independent Data Safety Monitoring Board (DSMB) consisting of at least 3 physicians and 1 statistician with appropriate scientific and medical expertise will review safety data from dose escalation approximately twice a year.

More details about study design can be found in the study protocol.

3.1.3. Overview of Plans for Data Analysis and Primary Clinical Study Report

The study data will be analyzed and reported in the primary clinical study report (CSR) at the time after all participants have completed 26 weeks of follow-up after CTX310 infusion or

discontinued earlier. Any additional data for subjects continuing in follow-up past the data cutoff date for the primary CSR will be included in the final analysis at the end of the study.

3.2 Study Measures and Endpoints

3.2.1. Safety Measures and Endpoints

Safety will be evaluated by the following:

- Reporting of adverse events (AEs), including determination of DLTs, serious adverse events (SAEs), treatment-related AEs, and AE of special interest (AESI). The severity of AEs will be assessed by the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) version 5.0. More details about grading for AE severity can be found in Section 9.4 of the study protocol. Frequency of adverse events by severity and relationship will be summarized, and the summaries will focus on treatment-emergent AEs (TEAEs), which refer to the AEs that started or worsened since the CTX310 infusion.
- Reporting of safety laboratory parameters and vital signs.

3.2.2. Efficacy Measures and Endpoints

The preliminary efficacy of CTX310 will be assessed by percentage changes in lipid levels over time compared to baseline.

3.2.3. PK/PD Measures and Endpoints

Pharmacokinetics (PK) of CTX310 will be assessed by plasma levels of LNP ([redacted] and [redacted]). Pharmacodynamics (PD) of CTX310 will be assessed by percentage change in ANGPTL3 concentration over time compared to baseline.

3.3 Sample Size Estimation

The sample size of the study will be approximately 69 subjects. This will include approximately 21 subjects in Phase 1a dose escalation and approximately 48 subjects in the Phase 1b disease-specific cohort expansion parts of this study. In Phase 1b, there will be up to 12 subjects per cohort.

No formal sample size calculation was performed for this study. The total sample size is estimated based on the dose escalation/expansion design and considered sufficient for the preliminary evaluation of the safety and tolerability of CTX310.

4. INTERIM ANALYSES

No formal efficacy interim analysis is planned.

The DSMB will review safety data as needed during the study to monitor stopping rules and to provide recommendations on enrollment or protocol amendment.

5. DEFINITIONS AND GENERAL METHODOLOGY

5.1 Definitions of Analysis Sets

5.1.1. Enrolled Set

All participants who sign the informed consent, meet the inclusion/exclusion criteria, and are enrolled in the study.

5.1.2. Safety Analysis Set

All participants who receive CTX310 infusion. Analyses of the safety assessments will be based on the Safety Analysis Set. The participants in the Safety Analysis Set will be classified by received CTX310 dose level.

5.1.3. Full Analysis Set

All participants who receive CTX310 infusion and have at least one post-baseline lipid assessments or discontinue earlier. The efficacy analyses will be performed based on the Full Analysis Set. Participants in the Full Analysis Set will be classified by received CTX310 dose level.

5.2 General Methodology

Disposition, demographic and baseline characteristics, exposure, efficacy, safety, and PK/PD data will be summarized.

Categorical data will be summarized by frequency distributions (number and percentages of participants) and continuous data will be summarized by descriptive statistics (number of participants [n], mean, standard deviation [SD], median, minimum, and maximum). All data will be provided in by-subject listings.

All analyses and summary tables will have the analysis set sample size (i.e., number of participants) for each dose level of CTX310 and overall in the column/row heading as applicable, unless specified otherwise.

All percentages, except for 100%, will be rounded to 1 decimal place. All mean and median values will be formatted to 1 more decimal place than the measured value. Standard deviation values will be formatted to 2 more decimal places. Minimum and maximum values will be presented to the same number of decimal places as raw data.

5.3 Randomization and Blinding

This is an open-label study with no randomization at enrollment. All participants are assigned to CTX310.

5.4 Baseline values

Unless otherwise specified, the baseline value is defined as the value collected at the time closest to, and prior to, the start of CTX310 infusion. Values collected at unscheduled visits prior to the start of CTX310 infusion will be included in the calculation of baseline values.

5.5 Method of Pooling Data

All data from all sites will be pooled. Study center or treatment-by-center interactions will not be included in any statistical analysis.

5.6 Handling of Missing Values

No imputation will be performed for missing values except for missing date (detailed in Appendix 9.2). For missing end date that could indicate an ongoing event (e.g., AEs and concomitant medication), the by-subject listings will include an indicator for ongoing status. Other missing data will be noted as missing.

No imputation will be performed for missing dates for by-subject listings. However, the imputation will be implemented for summary tabulations, when applicable. The details of the imputation methods for missing data in dates are provided in Appendix 9.2.

5.7 Relative Day since CTX310 Infusion

The date of initial CTX310 infusion will be considered relative day 1, and the day before the date of initial CTX310 infusion will be relative day -1. Relative days will be calculated as follows only when the full assessment date is known (i.e., partial dates will have missing relative days):

For days on or after the date of CTX310 infusion:

Date of Assessment – Date of CTX310 infusion + 1.

For days before the date of initial CTX10 infusion:

Date of Assessment – Date of CTX310 infusion.

5.8 Visit Windows

Whenever possible, the study assessments should occur on the scheduled visit day or specified time. Minor deviations from the scheduled visit day are allowed to accommodate participants' schedules (see Appendix 9.1 for schedule of assessments). In addition to protocol-mandated assessments, participants should be followed per institutional guidelines, and unscheduled assessments can be performed when clinically indicated. The assessments occurring at the scheduled visits are the primary assessments used for the statistical analyses. Other assessments falling outside the planned window will be included in worst toxicity grade derivation based on laboratory data, but not in descriptive statistics. All assessments, regardless of scheduled or unscheduled, will be included in by-subject listings.

5.9 Follow-up Durations

Potential follow-up duration for any participant treated with CTX310 will be calculated as the time interval from the CTX310 infusion to data cutoff date.

Actual follow-up duration for any participant treated with CTX310 will be calculated as the time interval from the first infusion of CTX310 to the last non-imputed date of record in the study database.

6. STATISTICAL ANALYSES

6.1 Subject Disposition

The number and percentage of participants in the following analysis sets will be summarized:

- Enrolled Set
- DLT Evaluable Set (DES)
- Safety Analysis Set (SAS)
- Full Analysis Set (FAS)

The percentage will be calculated based on the number of enrolled participants at each dose level and overall.

The number and percentage of participants in each disposition category below will be summarized with the number in the Safety Analysis Set (SAS) as the denominator:

- On study
- Completed study
- Discontinued from the study
    - Reason for discontinuation

Subject disposition data will be listed including CTX310 dose level, enrollment date, date of CTX310 infusion, status in study, study completion/discontinuation date, and reason for discontinuation as applicable.

Screen failures will be listed. For re-screened participants, enrollment status is based on the outcome of the last screening event.

6.2 Major Protocol Deviations

Major protocol deviations will be summarized for all treated participants in the Safety Analysis Set (SAS), including the number and percentage of participants with major protocol deviation.

By-subject listing for major protocol deviations will be provided.

6.3 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized using the SAS. Individual by-subject listings will be provided to support the summary tables.

6.3.1. Demographics

Age (years), baseline height (cm), baseline weight (kg), and baseline body mass index (BMI) will be summarized with descriptive statistics. Sex, ethnicity, and race will be summarized by frequency tabulations (count, percentage).

Body mass index will be calculated as: BMI (kg/m^2) = (weight in kg) / (height in m)^2.

6.3.2. Baseline Disease Characteristics

The following baseline characteristics of dyslipidemia will be summarized:

- Subtype of dyslipidemia (count and percentage):
    - Hypertriglyceridemia (HTG) (TG >150 mg/dL)

    - Moderate HTG (TG >150 mg/dL – 499 mg/dL)
    - Severe HTG (TG >=500 mg/dL)
  - Multifactorial chylomicronemia syndrome (MCS)
  - Homozygous familial hypercholesterolemia (HoFH)
  - Heterozygous familial hypercholesterolemia (HeFH)
  - Familial chylomicronemia syndrome (FCS)
  - Other HTG and/or hypercholesterolemia syndromes of undetermined etiologies
- Baseline lipid levels in serum (summary statistics):
  - Triglyceride (TG)
  - LDL-cholesterol (LDL-C)
  - Non-HDL-cholesterol (non-HDL-C)
  - HDL-cholesterol (HDL-C)
  - ApoB
  - ApoCIII
  - Remnant cholesterol

The central lab test results of lipid levels will be used for the analyses. The lipid levels are directly available as lab test results except for remnant cholesterol, which will be calculated as follows:

Remnant Cholesterol = Total Cholesterol – LDL-C – HDL-C.

6.4 Prior and Concomitant Medications/Procedures

All medications taken within 30 days before the signing of the informed consent form (ICF) will be recorded. All concurrent therapies, including prescription and nonprescription medications, will be recorded from the date of signed ICF through 12 months after CTX310 infusion.

Prior and concomitant medications/procedures will be coded using the World Health Organization (WHO) Drug Dictionary. Missing date imputation rules are included in Appendix 9.2.

6.4.1. Prior and Concomitant Lipid-lowering Therapies

Categorical summary (count and percentage of participants) will be provided for the common lipid-lowering therapies, including statin, ezetimibe, PCSK9 inhibitor, fibrates, ANGPTL3 targeted agent, lomitapide, omega-3 fatty acid, niacin, icosapent ethyl, bile acid sequestrants, and plasma apheresis.

All prior and concomitant lipid-lowering therapies will be listed.

6.4.2. Prior and Concomitant Medications/procedures other than lipid-lowering therapies

Concomitant medications other than lipid-lowering therapies will be tabulated descriptively by WHO Drug Dictionary anatomical therapeutic chemical (ATC) and preferred term (PT). They will also be listed. Concomitant procedures will be listed.

6.5 Medical History

Medical Dictionary for Regulatory Affairs (MedDRA) will be used to code medical history, including system organ class (SOC) and preferred term (PT).

Medical history will be listed in a by-subject listing.

6.6 Extent of Exposure and Compliance to Study Treatment

Study treatment refers to CTX310 infusion.

Summary of exposure to CTX310 will include actual dose normalized by eLBW (mg/kg) and total dose of RNA (mg). Descriptive statistics will be provided by CTX310 dose level. The LBW will be estimated using the following formula (Janmahasatian et al):

Female: eLBW (kg) = (9270 * [total body weight in kg]) / (8780 + (244 * [BMI in kg/m^2])),

Male: eLBW(kg) = (9270 *[total body weight in kg]) / (6680 + (216 * [BMI in kg/m^2])),

where BMI (kg/m^2) = (total body weight in kg) / (height in m)^2.

Total dose of RNA will be calculated using the following formula:

Total dose of RNA (mg) = RNA concentration (mg/mL) * volume administered (mL),

where both RNA concentration and volume are before dilution, and the volume administered takes in consideration of a small volume (estimated to be ~1.1mL) trapped in filter during preparation.

The number and percentage of participants with the following type of dose modification will also be summarized:

- Infusion decreased, by reason.
- Infusion interrupted.

By-subject listing of the exposure to CTX310 will be provided.

6.7 Efficacy and Pharmacodynamics Analyses

The preliminary efficacy of CTX310 for refractory dyslipidemia will be assessed based on the Full Analysis Set (FAS). The efficacy and pharmacodynamics (PD) endpoints of percentage change in lipid and ANGPTL3 concentration over time compared to baseline will be summarized using descriptive statistics (mean, standard deviation, min and max) for each dose level. The summaries will be provided for the scheduled timepoints as specified in the protocol.

The lipids for efficacy assessment include:

- Triglyceride (TG)
- Apolipoprotein C-III (ApoCIII)
- Low-density-lipoprotein cholesterol (LDL-C)
- Apolipoprotein B (ApoB)
- Non-high-density-lipoprotein cholesterol (non–HDL-C)
- High-density-lipoprotein cholesterol (HDL-C)

The analyte for PD assessment includes:

- Angiopoietin-like 3 (ANGPTL3)

The central lab test results will be used for the analyses, unless otherwise specified. The lipid levels are directly available as lab test results. If the concentration of an analyte is below the lower limit of quantification (LLOQ), the half of LLOQ will be used as the imputed concentration value.

Plots to depict the mean percentage changes with standard error of mean (SEM) over time compared to baseline will be provided for each analyte by dose level.

Efficacy at the lowest CTX310 dose levels 1 and 2 (0.1 and 0.3 mg/kg eLBW) will be analyzed separately and pooled.

The percentage changes in lipids (TG, ApoCIII, LDL-C, ApoB, non-HDL-C, HDL-C) will also be summarized for participants who received dose levels 3 or higher (>=0.6mg/kg eBLW) in the following subgroups:

- Participants with at least moderately elevated TG levels (>150 mg/dL) at baseline
- Participants with severe elevations in TG levels (>=500 mg/dL) at baseline
- Secondary prevention participants with LDL-C >70 mg/dL at baseline
- Primary prevention participants with LDL-C >100 mg/dL at baseline

In addition to the descriptive summaries (mean, standard deviation, min and max), categorical summaries (count and percentage) for responders will be provided for participants who received dose levels 3 or higher (>=0.6mg/kg eBLW). The following responder categories will be included:

- For participants with elevated TG (>150mg/dL) at baseline, the proportion of responders whose post-baseline TG level ever reached below 150mg/dL.

  The following subgroup analyses based on the severity of hypertriglyceridemia (HTG) at baseline will be performed if greater than n=3 participants are available per group:

  - Severe HTG with baseline TG >= 500 mg/dL who respond to <500 mg/dL
  - Severe HTG with baseline TG >= 500 mg/dL who respond to < 150 mg/dL
  - Progressive Chylomicronemia with TG > 880 mg/dL who respond to <500 mg/dL
  - Progressive Chylomicronemia with TG > 880 mg/dL who respond to <150 mg/dL
- For participants with elevated LDL-C, depending on whether they had prior ASCVD events (primary prevention if no prior ASCVD events, secondary if yes) there are two subcategories to determine the proportion of responders:
  - Among secondary prevention participants with LDL-C > 70 mg/dL at baseline, the responders whose post-baseline LDL-C level ever reached below 70 mg/dL.
  - Among primary prevention participants with LDL-C > 100 mg/dL at baseline, the responders whose post-baseline LDL-C level ever reached below 100 mg/dL.

6.8 Safety and Tolerability

The safety-related information of all participants enrolled in this study will be recorded from the time of ICF signing until the end of study; however, there are different AE reporting requirements for different time periods in the study (Section 9.7 in the study protocol).

The primary analysis of safety data will summarize adverse events (AEs) and laboratory values based on safety analysis set, i.e., all the participants who receive CTX310 infusion.

Safety data will be summarized by CTX310 dose level and overall. All safety data collected since the time of ICF will be listed.

Adverse events (AEs) will be coded with the Medical Dictionary for Regulatory Activities (MedDRA) at the time of analysis. The version of the MedDRA may vary over time as the current version in use is updated. The severity of adverse events will be graded using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5.0. More details on AE severity grading can be found in Section 9.4 of the study protocol.

6.8.1. Dose-Limiting Toxicities

The DLT Evaluable Set (DES) will be used as the analysis set during dose escalation to determine the RP2D. DLTs are defined in the study protocol (Section 5.4.3). The DLT evaluation period will begin with the CTX310 infusion and last for 30 days. If a DLT-evaluable participant (i.e., a participant that has been administered CTX310, and has completed the 30-day DLT evaluation period) has signs or symptoms of a potential DLT, the DLT evaluation period will be extended according to the protocol-defined window to allow for improvement or resolution before a DLT is declared. Number and percentage of participants who experienced DLTs will be summarized by CTX310 dose level and overall. By-subject listings will be provided for DLTs.

6.8.2. Adverse Events

Summaries of AEs will focus on treatment-emergent AEs (TEAEs), which refer to the AEs that started or worsened since the CTX310 infusion. Safety Analysis Set will be used as the analysis set for TEAEs. The incidence of TEAEs will be summarized according to MedDRA by system organ class (SOC) and/or preferred term (PT), CTCAE grade, and relation to CTX310 treatment. If a subject experiences multiple AEs under the same PT within a SOC, the subject will be counted only once for that PT within that SOC. If a subject experienced the same AE more than once with different grades, the event with the highest grade will be tabulated in by-grade tables.

Tables summarizing the incidence of TEAEs include:

- Overall summary of TEAEs
- All TEAEs by PT, in order of frequency among overall participants
- AESI by grade
- All TEAEs by SOC and PT
- TEAEs related to CTX310 by SOC and PT
- Grade 3 or higher TEAEs by SOC and PT
- Grade 3 or higher TEAEs related to CTX310 by SOC and PT
- Serious AEs (SAEs) by SOC and PT
- Serious AEs (SAEs) related to CTX310 by SOC and PT
- TEAEs leading to death by SOC and PT

For overall summary of TEAEs, the following categories will be included:

- Participants with any TEAE
- Participants with grade ≥3 TEAE
- Participants with TEAE related to CTX310
- Participants with grade ≥3 TEAE related to CTX310
- Participants with AESI
- Participants with Grade ≥3 AESI
- Participants with serious TEAE
- Participants with serious TEAE related to CTX310
- Participants with TEAE leading to death
- Participants with DLTs

Summary tables based on broad term, including the ones selected by Standardized MedDRA Query (SMQ) search criteria, will be provided for applicable AEs at the discretion of medical monitor.

In addition to the summary tables, by-subject AE listings for SAEs and AEs leading to death will be provided for participants in Safety Analysis Set.

By-subject AE listings for all AEs will be provided for all participants in Enrolled Set.

6.8.3. Adverse Events of Special Interest

An adverse event of special interest (AESI) refers to an AE of scientific and medical concern specific to the sponsor's product or program for which ongoing monitoring and rapid communication by the investigator to the sponsor may be appropriate (Section 9.3 in the study protocol). Number and percentage of participants who experienced AESI will be summarized for each grade (1-5), using Safety Analysis Set by CTX310 dose level and overall.

The following AESI categories will be presented:

- Infusion-related reactions (IRRs).
- Abnormal coagulation findings, defined as clinically relevant abnormal bleeding, thrombotic, or hemorrhagic events.
- Increase in ALT or AST: ≥3 × ULN or ≥2 × the baseline value (if baseline ALT ≥ULN).
- Allergic reactions/events or localized reactions (collected for 30 days post-infusion).
- New malignancy.

6.8.4. Death

Death listing will be provided for all participants in Safety Analysis Set. The death date and the relative day since the CTX310 infusion will be displayed if applicable.

6.8.5. Clinical Laboratory Evaluations

Laboratory samples for safety assessment will be collected and analyzed according to the schedule of assessments as specified in the study protocol. Unless stated, local laboratories meeting country-specific requirements for clinical testing will be utilized to analyze all tests. For laboratory tests covered by the CTCAE version 5.0, laboratory results will be graded accordingly. A Grade 0 will be assigned for all non-missing values not graded as 1 or higher. For laboratory tests where grades are not defined by CTCAE, results will be graded by the low/normal/high classifications based on laboratory normal ranges. If a lab value is reported using a non-numeric qualifier (e.g., less than [<] a certain value, or greater than [>] a certain value), the given numeric value will be used in the summary statistics, ignoring the non-numeric qualifier.

The following laboratory parameters will be summarized in tabulation:

- Hematology: Hematocrit, hemoglobin, red blood cell count, white blood cell count and platelet count. From the complete blood count differential both the absolute and percent of neutrophils, lymphocytes, monocytes, basophils and eosinophils will be analyzed if data are available.
- Serum Chemistry: ALT (SGPT), AST (SGOT), bilirubin (total and direct), total protein, albumin, alkaline phosphatase, bicarbonate, BUN, calcium, chloride, creatinine, eGFR (MDRD equation), glucose, magnesium, phosphorus, potassium, sodium, HbA1c, C-reactive protein

Summary tables with descriptive statistics for the actual values and changes from baseline of the clinical laboratory parameters over time will be provided. Safety Analysis Set will be used for the summaries.

Besides the common analyses on clinical laboratory parameters, additional analyses will be performed on the parameters related to liver chemistry tests (Section 7.1.2 of the study protocol). Specifically, the maximum values of ALT, AST and total bilirubin will be summarized with descriptive statistics by CTX310 dose level and overall. They will also be summarized categorically with the following categories:

- >3 × ULN or >2 × the baseline value (if baseline ALT >ULN) for ALT and AST
- >5 × ULN for ALT and AST
- >8 × ULN for ALT and AST

In addition to the summary tables, the listings of hematology and serum chemistry laboratory data with the corresponding CTCAE grades or the classifications relative to the laboratory normal ranges will be provided for all participants in Safety Analysis Set.

The following lab test results will be listed but not summarized:

- Urinalysis: Specific gravity, pH, glucose, protein, blood, ketones, bilirubin, urobilinogen, nitrite, leukocyte esterase, microscopic examination (if blood or protein is abnormal)
- Coagulation: prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (aPTT), fibrinogen
- Pregnancy test: hCG

- Viral serology: HIV-1, HIV-2, hepatitis C virus (HCV) antibody and RNA, hepatitis B virus (HBV) surface antigen, HBV surface antibody, HBV core antibody
- Thyroid function: T3, T4, TSH

6.8.6. Vital Signs

Vital sign measurements will be listed for all participants in Safety Analysis Set.

6.8.7. Electrocardiograms (ECGs)

Twelve (12)-lead electrocardiograms (ECGs) will be obtained per schedule of assessments as specified in the study protocol. Both the numeric values and assessments interpreted as normal, abnormal not clinically significant, abnormal clinically significant are collected.

All ECG values along with the categorical interpretation will be presented in the listings by subject and visit time point for all participants in Safety Analysis Set.

6.9 Exploratory Analyses

6.9.1. Percentage change in liver fat fraction from baseline

A liver MRI–PDFF (for assessment of fatty liver/steatosis) is performed at screening and end of study (EOS) visits for the majority of participants. Percentage change in MRI-PDFF based hepatic fat fraction (HFF) at EOS from baseline will be summarized using descriptive statistics (mean, standard deviation, min, max) by CTX310 dose level. Clinically significant findings reported as medical history or AEs will be included in the categorical summaries (count and frequency) for medical history or AEs, respectively.

Subgroup analysis will be performed on the participants with elevated HFF > 8% using MRI-PDFF at baseline, with percentage changes in HFF summarized using descriptive statistics in this subgroup.

6.9.2. Percentage change in remnant cholesterol from baseline

Remnant cholesterol will be calculated using the following formula:

$$\text{Remnant Cholesterol} = \text{Total Cholesterol} - \text{LDL-C} - \text{HDL-C},$$

where LDL-C and HDL-C refer to low density lipoprotein (LDL) cholesterol and high density lipoprotein (HDL) cholesterol, respectively.

Percentage changes in remnant cholesterol over time from baseline will be summarized using descriptive statistics by CTX310 dose level.

7. COMPUTER SOFTWARE

SAS Version 9.4 or later will be used to perform the analyses and produce the specified tables, listings, and figures.

8. REFERENCES

Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet. 2005;44:1051–1065.

9. APPENDIX

9.1 Schedule of Assessments

		Treatment	Follow-up											
	Screening[1]	Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	**D -42 to -1**	**D1**	**D2**	**D3**	**D4**	**W1 D7 ±1d**	**W2 D14 ±2d**	**W3 D21 ±4d**	**D30 ±4d**	**M2/ D60 ±7d**	**M3/ D90 ±7d**	**M6/ D180 ±7d**	**M9/ D270 ±14d**	**EOS/ M12/ D360 ±14d[3]**
Eligibility and Other Assessments														
Informed consent	X													
Demographics, medical history	X													
History of pancreatitis events (for subjects with severe HTG)[4]	X													
Physical exam[5]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height, weight, BMI, waist to hip ratio	X													X
Vital signs[6]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Liver MRE or FibroScan[7]	X													
Liver MRI-PDFF or ultrasound[7]	X													X
Echocardiogram[8]	X[7]													
12-lead ECG[9]	X	X	X			X			X		X	X		X
Eligibility confirmation[10]	X													

	Screening[1]	Treatment				Follow-up								
		Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	**D -42 to -1**	**D1**	**D2**	**D3**	**D4**	**W1 D7 ±1d**	**W2 D14 ±2d**	**W3 D21 ±4d**	**D30 ±4d**	**M2/ D60 ±7d**	**M3/ D90 ±7d**	**M6/ D180 ±7d**	**M9/ D270 ±14d**	**EOS/ M12/ D360 ±14d[3]**
Treatment														
Pre-infusion prophylaxis regimen[11]	X	X												
CTX310 infusion[12]		X												
Safety Assessments														
Acute DLT assessment		X	X	X	X	X	X	X	X					
Adverse events	X													
Concomitant meds	X													
Laboratory Assessment (Local)[13]														
Serum chemistry	X	X	X	X	X	X	X	X	X		X	X	X	X
Urinalysis	X		X		X				X		X	X	X	X
Coagulation panel	X	X	X	X	X	X	X	X	X		X	X	X	X
Pregnancy test[14]	X													X
Hematology (CBC)	X	X	X	X	X	X	X		X			X		X
HbA1c	X											X	X	X
Thyroid function	X											X		X
eGFR (MDRD equation)	X								X			X		X
Viral serology	X													
Cardiac biomarker test[13]		X												

		Treatment		Follow-up										
	Screening[1]	Inpatient Hospitalization[2]		Daily Safety Visits										
Assessment	D -42 to -1	D1	D2	D3	D4	W1 D7 ±1d	W2 D14 ±2d	W3 D21 ±4d	D30 ±4d	M2/ D60 ±7d	M3/ D90 ±7d	M6/ D180 ±7d	M9/ D270 ±14d	EOS/ M12/ D360 ±14d[3]
Laboratory Assessments (Central)														
Genetic testing[15]	X													
Lipid panel[16]	X						X		X	X	X	X	X	X
Biomarkers (Plasma, Central)[17]														
ANGPTL3 levels[18]	X						X		X		X	X	X	X
PK studies[19]	X	X	X	X	X	X	X		X		X	X		X
Exploratory Biomarkers (Central)														
Immunogenicity[20]	X					X			X		X	X		X
Whole blood for storage[21]	X													
Plasma for storage[22]	X													
Serum[23]	X	X	X	X										

AESI: adverse event of special interest; ANGPTL3: angiopoietin-like 3; BMI: body mass index; Cas9: CRISPR-associated protein; CBC: complete blood count; CRISPR: clustered regularly interspaced short palindromic repeats; D or d: day; DLT: dose-limiting toxicity; ECG: electrocardiogram; eGFR: estimated glomerular filtration rate; eLBW: estimated lean body weight; EOS: end of study; HbA1c: glycosylated hemoglobin; HTG: hypertriglyceridemia; LTFU: long-term follow-up; M: month; meds: medications; MDRD: Modification of Diet in Renal Disease; MRE: magnetic resonance elastography; MRI-PDFF: magnetic resonance imaging–protein density fat fraction; PK: pharmacokinetic; W: week.

[1] See study protocol Section 8.1.1.1 for detailed guidance. For the subject's convenience, if an assessment was performed before signing the ICF as part of the subject's routine clinical evaluation, it does not need to be repeated if the testing fulfills the study requirements and is performed within the screening timeframe. Subjects will be allowed a one-time rescreening, which may take place within 3 months of the initial consent. All screening tests should be

repeated to determine eligibility except for genotyping (if performed during the initial screening) and FibroScan/MRE of liver, MRI-PDFF/ultrasound of liver, and infectious disease markers (if these procedures were performed within 60 days prior to infusion).

[2] Subjects will be hospitalized on Day 1 for CTX310 infusion. Hospital discharge will occur a minimum of 24 hours post completion of CTX310 infusion and after Day 2 safety evaluations are complete and relevant laboratory tests have been reviewed. Subjects must remain in the geographic area (staying within 1 hour of the infusion site) to allow for daily and as needed clinic visits for safety assessment until completion of the Day 4 visit. Inpatient hospitalization may be extended beyond 24 hours completion of CTX310 infusion, or following hospital discharge, the subject may be readmitted or required to stay in the geographic area beyond Day 4 at the discretion of the investigator as needed for safety monitoring or if required by local regulation or site practice. See study protocol Section 6.1.2 for discharge criteria and study protocol Section 8.1.1 for further description of study periods.

[3] All subjects who receive an infusion of CTX310, including those who discontinue the study prior to 12 months, will be asked to consent to roll over into a separate LTFU study for up to 15 years post-infusion (study protocol Section 8.1.1.5).

[4] Among subjects enrolled in Phase 1b with severe HTG, the number of episodes of acute pancreatitis within 12 months prior to enrollment and during the screening period will be collected (study protocol Section 8.2.2).

[5] Complete physical exam required at screening, D1, D30, and EOS visits. Abbreviated symptom-targeted physical exam may be performed at all other time points (study protocol Section 8.2.3).

[6] Vital signs: Blood pressure, heart rate, respiratory rate, oxygen saturation, temperature (study protocol Section 8.2.4). Subjects excluded from the study due to uncontrolled hypertension must have blood pressure measurements repeated at least 15 minutes later for confirmation. On Day 1 vital signs should be recorded at the following time points: prior to pre-infusion prophylaxis, prior to the infusion of CTX310, every 15 (± 5) minutes) during the infusion, at 1, 2, 3, 6 hours (± 15 minutes), and 8 hours (± 30 minutes) after the end of the infusion, and then every 8 hours (± 30 minutes) until discharge from the hospital.

[7] Liver imaging: The same type of imaging should be used across all study visits. See study protocol Section 8.2.5.

[8] Transthoracic echocardiogram will be performed at screening. See study protocol Section 8.2.6 for details.

[9] Electrocardiogram: See study protocol Section 8.2.7. Day 1 ECG can be collected within 24 hours prior to Day 1 pre-infusion prophylaxis regimen.

[10] Study eligibility must be determined by the investigator and confirmed by the medical monitor (study protocol Section 8.1.1.1) prior to inpatient admission for study treatment. Also See Laboratory Assessments for Eligibility Confirmation (study protocol Section 8.2.8).

[11] Pre-infusion prophylaxis regimen: See study protocol Section 6.1.1.

[12] See study protocol Section 14.1 for eLBW calculation. See study protocol Section 6.1 for other details regarding CTX310 administration.

[13] Day 1 local laboratory assessments should be performed within 24 hours prior to the infusion of CTX310. Prior to the administration of CTX310 on Day 1, elevation in cardiac biomarker, troponin I or T, requires discussion with the sponsor's medical monitor. On all other days, serum chemistry may be repeated as required as per study protocol Section 7.1.2 to monitor AESI (study protocol Section 9.3) and stopping rules (study protocol Section 10.2).

[14] At screening, all females must have a negative serum pregnancy test performed as per local standard. Serum or urine pregnancy test can be performed at EOS/M12 Visit. See study protocol Section 8.2.8, Table 4, and study protocol Section 8.2.9.

[15] The sample for genetic testing by the central lab should be collected during screening prior to CTX310 infusion. See study protocol Section 8.2.8 and Table 5 for details. Collection of a sample for genetic testing should not be repeated during rescreening, if applicable

[16] Lipid panel: See study protocol Section 8.2.8, Table 5 for listing of lipid panel components. Subjects on apheresis should have lipid levels sampled within 5 days prior to the procedure (pre-apheresis sample on the day of apheresis is also adequate). All lipid panels must be performed after a minimum 8 hour fast.

[17] Sponsor may request discontinuation of sample collections. Continue sample collection for all listed time points until instructed otherwise by the sponsor.

[18] Plasma samples will be obtained to assess ANGPTL3 levels (study protocol Section 8.4.2).

[19] PK testing: See study protocol Section 8.4.1: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (± 15 min) hours after completion of CTX310 infusion. On D2, D3, and D4 (24 [± 2], 48 [± 2], and 72 [± 2] hours post-infusion time points, respectively), a single sample will be collected. A single sample will also be collected for all other scheduled time points.

[20] Immunogenicity: See study protocol Section 8.3.

[21] Whole blood collection: Whole blood samples will be obtained at screening and stored (study protocol Section 8.4.3.1).

[22] Plasma for storage: See study protocol Section 8.4.3.2.

[23] Serum samples for exploratory biomarker assessments (e.g., cytokines): See study protocol Section 8.4.3.3: On D1, samples should be collected prior to infusion, within 5 minutes post-CTX310 infusion, and at 1 (± 5 min), 2 (± 5 min), and 7 (±15 min) hours after completion of CTX310 infusion. On D2 and D3 (24 [± 2] and 48 [± 2] hours post-infusion time points, respectively), a single sample will be collected.

9.2 Imputation Methods for Missing Data in Dates

9.2.1. Missing/Partial Dates in Adverse Events

Missing/Partial Start Date:

1. *Missing day only*
   - If the month and year are the same as that of Day1 (Date of 1st dose of CTX310), Day1 will be assigned
   - If the month and year are before that of Day1, the last day of the month will be assigned
   - If the month and year are after that of Day1, the first day of the month will be assigned
2. *Missing day and month*
   - If the year is the same as that of Day1, Day1 will be assigned
   - If the year is before that of Day1, December 31 will be assigned
   - If the year is after that of Day1, January 1st will be assigned
3. *Missing day, month, and year*
   - Day1 will be assigned

If the stop date is non-missing and the imputed start date is after the stop date, the stop date will be used as the start date.

Missing/Partial Stop Date:

1. *Missing day only*
   - The last day of the month will be assigned as the missing day.
2. *Missing day and month*
   - December 31 will be assigned to the missing fields.
3. *Missing day, month and year*
   - If event is resolved but stop date is complete missing, then end of study date or data cut off data whichever is earlier will be assigned.

If the start date is non-missing and the imputed stop date is before the start date, the start date will be used as stop date. If the death date is available and the imputed stop date is after the death date, the death date will be used.

9.2.2. Missing/Partial Dates in Prior or Concomitant Lipid-lowering Therapies

Missing/Partial Start or Stop Date:

1. *Missing day only*
   - 15th of the month
2. *Missing day and month*
   - 30th of June
3. *Missing day, month, and year*

- No imputation will be applied

If the stop date is non-missing and the imputed start date is after the stop date, the stop date will be used as the start date. If the start date is non-missing and the imputed stop date is before the start date, the start date will be used as stop date.

John Baker

Oct 15, 2025 3:31 PM ET
New England Journal of Medicine

Discloser Identifier: 1270926
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Peter Clifton

Oct 07, 2025 5:28 PM ET
New England Journal of Medicine

Discloser Identifier: 1270924
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Jason Duran

Discloser Identifier: 1270936
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   Yes.

   a. **Please describe those relationships.**

      I am a full time employee and shareholder at CRISPR Therapeutics AG

2. **What is the manuscript title?**

   Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Luke Laffin

Oct 07, 2025 11:22 AM ET
New England Journal of Medicine

Discloser Identifier: 1234793
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
AstraZeneca	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
Cedars-Sinai Medical Center	Other	Self
Category: Other **Description:** Speaking Engagement **Additional Information:**		
Eli LIlly	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
Idorsia	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
LucidAct Health Inc	Stock Option	Self
Additional Information:		
Medtronic	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
Novartis	Consultant	Self
Category: Consultant **Description:** Steering committee for clinical trials. **Additional Information:**		
Novo Nordisk	Consultant	Self
Category: Consultant **Description:** Payment via IQVIA **Additional Information:**		
Recor	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
Ripple Medical	Consultant	Self

Entity	Type	Interest Held By
Category: Consultant **Description:** **Additional Information:**		
Stability Health	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		
University of Chicago	Other	Self
Category: Other **Description:** Speaking Engagement **Additional Information:**		
Veradermics Inc	Consultant	Self
Category: Consultant **Description:** **Additional Information:**		

Intellectual Property

Type		Is Licensed	Interest Held By
Other Intellectual Property - Royalties for book / editor (Springer)		-	Self
Description: Royalties for book / editor (Springer) **Additional Information:** **Licensees:** No **Additional Information:**	**Type:** Book		
Other Intellectual Property - Royalties for book / editor (Elsevier)		-	Self
Description: Royalties for book / editor (Elsevier) **Additional Information:** **Licensees:** No **Additional Information:**	**Type:** Book editing		
Other Intellectual Property - Royalties for book / editor (Cleveland Clinic, Be		-	Self
Description: Royalties for book / editor (Cleveland Clinic, Belvoir Media) **Additional Information:** **Licensees:** No **Additional Information:**	**Type:** Cleveland Clinic Heart Advisor		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certi cation

I certify that the information provided in this disclosure is complete and accurate.

Stephen Nicholls

Oct 07, 2025 9:57 AM ET
New England Journal of Medicine

Discloser Identifier: 905292
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
Amarin Pharma Inc.	Consultant	Self

Category: Consultant
Description: consultant
Additional Information: consultant

Amgen	Grant / Contract	Other - Organisation

Recipient Name: SAHMRI and Monash University
Recipient Type: Institution
Grant / Contract Description: Clinical trial
Grant / Contract Purpose: Research
Additional Information:

Anthera	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution
Grant / Contract Description: Clinical trial
Grant / Contract Purpose: Research
Additional Information:

Arrowhead Pharmaceuticals	Consultant	Self

Category: Consultant
Description:
Additional Information: Consultant fees paid to institution

AstraZeneca	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

Boehringer Ingelheim	Grant / Contract	Self

Recipient Name: Monash University
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

Cerenis	Grant / Contract	Other - Organisation

Recipient Name: SAHMRI
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

CSL Sequiris	Consultant	Self

Category: Consultant
Description: Paid to institution
Additional Information:

Cyclarity	Grant / Contract	Self

Recipient Name: Monash University
Recipient Type: Institution
Grant / Contract Description:
Grant / Contract Purpose: Research
Additional Information:

Entity	Type	Interest Held By
Daiichi Sankyo Company	Consultant	Self

Category: Consultant
Description: Paid to institution
Additional Information:

Eli Lilly and Company	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic and Monash University
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

Esperion	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

F. Hoffmann-La Roche	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

InfraReDx	Grant / Contract	Other - Organisation

Recipient Name: SAHMRI
Recipient Type: Institution
Grant / Contract Description: core lab
Grant / Contract Purpose: Research
Additional Information:

Kinkisa Pharmaceuticals	Consultant	Self

Category: Consultant
Description: Paid to institution
Additional Information:

LipoScience	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution
Grant / Contract Description: analysis
Grant / Contract Purpose: Research
Additional Information:

New Amsterdam Pharma	Grant / Contract	Other - Institution

Recipient Name: Monash University
Recipient Type: Institution
Grant / Contract Description: clinical trial
Grant / Contract Purpose: Research
Additional Information:

Novartis	Grant / Contract	Other - Organisation

Recipient Name: Monash University
Recipient Type: Institution
Grant / Contract Description: trial
Grant / Contract Purpose: Research
Additional Information:

Novo Nordisk	Consultant	Self

Category: Consultant
Description:
Additional Information:

Resverlogix	Grant / Contract	Other - Organisation

Recipient Name: Cleveland Clinic
Recipient Type: Institution

Entity	Type	Interest Held By
Grant / Contract Description: trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Sanofi-Regeneron	Grant / Contract	Other - Organisation
Recipient Name: SAHMRI	**Recipient Type:** Institution	
Grant / Contract Description: trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Scribe Therapeutics	Consultant	Self
Category: Consultant		
Description:		
Additional Information: honoraria paid to institution		
The Medicines Company	Grant / Contract	Other - Organisation
Recipient Name: Cleveland Clinic	**Recipient Type:** Institution	
Grant / Contract Description: trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Vaxxinity	Grant / Contract	Other - University
Recipient Name: Monash University	**Recipient Type:** Institution	
Grant / Contract Description: trial	**Grant / Contract Purpose:** Research	
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Jennifer Nielsen

Oct 08, 2025 1:01 PM ET
New England Journal of Medicine

Discloser Identifier: 1270934
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
CRISPR Therapeutics	Employment	Self

Title: Senior Vice President, in vivo portfolio
Position Description:
Additional Information:

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3 (25-11778)

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Steven Nissen

Oct 07, 2025 9:51 AM ET
New England Journal of Medicine

Discloser Identifier: 7234
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
AbbVie	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: TRAVERSE clinical trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Amgen	Consultant	Self
Category: Consultant		
Description: advice regarding PCSK9 inhibitor		
Additional Information:		
Amgen	Consultant	Self
Category: Consultant		
Description: advice regarding PCSK9 inhibitor		
Additional Information:		
Arrowhead Pharmaceuticals	Grant / Contract	Self
Recipient Name: C5 Research	**Recipient Type:** Institution	
Grant / Contract Description: Support to conduct a clinical trial	**Grant / Contract Purpose:** Research	
Additional Information:		
AstraZeneca	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: TACTIC clinical trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Bristol-Myers Squibb	Grant / Contract	Self
Recipient Name: C5 Research	**Recipient Type:** Institution	
Grant / Contract Description: VALOR Clinical Trial ODYSSEY Clinical Trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Cleveland Clinic	Employment	Self
Title: Chief Academic Officer, Heart Vascular and Thoracic Institute	**Position Description:** In charge of all academic activities with this institute	
Additional Information:		
CRSPR Therapeutics	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: Clinical trial development	**Grant / Contract Purpose:** Research	
Additional Information:		
Eli LIlly	Travel	Self
Location(s): Indianapolis		
Purpose: clinical trial		
Additional Information:		

Entity	Type	Interest Held By
Eli Lilly and Company	Grant / Contract	Self
Recipient Name: C5 Research	**Recipient Type:** Institution	
Grant / Contract Description: SURMOUNT MMO clinical trial ACCLAIM Clinical Trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Esperion Therapeutics, Inc.	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: CLEAR Outcomes Trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Glenmark Pharmaceuticals Limited	Consultant	Self
Category: Consultant		
Description: generic rosuvastatin		
Additional Information:		
Kardigan	Other	Self
Category: Other		
Description: Executive Committee Chair for two clinical trials		
Additional Information: No compensation		
Mineralys	Grant / Contract	Self
Recipient Name: C5 Research	**Recipient Type:** Institution	
Grant / Contract Description: Funding for academic research organization to conduct clinical trials	**Grant / Contract Purpose:** Research	
Additional Information: no personal reimbursement		
New Amsterdam Pharmaceuticals	Grant / Contract	Self
Recipient Name: C5 Research	**Recipient Type:** Institution	
Grant / Contract Description: Funding for Academic Research Organization (C5 Research) to conduct a Phase 3 Trial	**Grant / Contract Purpose:** Research	
Additional Information: No personal reimbursement		
Novartis	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: Lp(a) Horizon clinical trial	**Grant / Contract Purpose:** Research	
Additional Information:		
Silence Pharmaceuticals	Grant / Contract	Self
Recipient Name: C5Research	**Recipient Type:** Institution	
Grant / Contract Description: APOLLO clinical trial ALPACAR Clinical Trial	**Grant / Contract Purpose:** Research	
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   Tirzepatide, Dulaglutide, and Cardiovascular Outcomes in High Cardiovascular Risk Patients with Type 2 Diabetes

3. **Are you the corresponding author?**

No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Naimish Patel

Oct 13, 2025 10:39 AM ET
New England Journal of Medicine

Discloser Identifier: 1270935
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
CRISPR Therapeutics AG	Employment	Self

Title: Chief Medical Officer

Position Description: In charge of all compounds in clinical development pipeline and all Development teams advancing these compounds.

Additional Information:

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   Yes.

   a. **Please describe those relationships.**

      I am an employee of CRISPR Therapeutics AG

2. **What is the manuscript title?**

   Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Ashish Sarraju

Oct 07, 2025 10:46 AM ET
New England Journal of Medicine

Discloser Identifier: 1117989
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
New Amsterdam Pharma	Other	Self

Category: Other

Description: Principal Investigator

Additional Information: PI for clinical trial. No personal compensation. Research funding paid to institution for academic research organization activities.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Russell Scott

Oct 07, 2025 12:14 PM ET
New England Journal of Medicine

Discloser Identifier: 1101743
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Shweta Singh

Oct 15, 2025 8:46 AM ET
New England Journal of Medicine

Discloser Identifier: 1270931
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
CRISPR Therapeutics AG	Employment	Self
Title: Director, Clinical Development	**Position Description:** Clinical Scientist	
Additional Information:		
CRISPR Therapeutics AG	Stock	Self
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First in Human Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Convey Global Disclosure System | AAMC

Qiuqing Wang

Discloser Identifier: 732454
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Katherine Wolski

Oct 07, 2025 10:59 AM ET
New England Journal of Medicine

Discloser Identifier: 336558
Disclosure Purpose: 25-11778

Summary of Interests

I do not have any interests to disclose at this time.

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 gene editing targeting ANGPLT3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Huansheng Xu

Oct 08, 2025 9:09 PM ET
New England Journal of Medicine

Discloser Identifier: 1270933
Disclosure Purpose: 25-11778

Summary of Interests

Company or Organization

Entity	Type	Interest Held By
CRISPR Therapeutics AG	Employment	Self
Title: Director, Biostatistics	**Position Description:**	
Additional Information:		
CRISPR Therapeutics AG	Stock	Self
Additional Information:		
CRISPR Therapeutics AG	Stock Option	Self
Additional Information:		

Additional Questions

1. **Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?**

   No.

2. **What is the manuscript title?**

   First-in-Human Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3

3. **Are you the corresponding author?**

   No.

Certification

I certify that the information provided in this disclosure is complete and accurate.

Data Sharing Statement

Laffin LJ, Nicholls SJ, Scott RS, et al. Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3. N Engl J Med. DOI: 10.1056/NEJMoa2511778.

Question	Authors' Response
Will the data collected for your study be made available to others?	No
Would you like to offer context for your decision?	—
Which data?	—
Additional information about data	—
How or where can the data be obtained?	—
When will data availability begin?	—
When will data availability end?	—
Will any supporting documents be available?	—
Which supporting documents?	—
Additional information about supporting documents	—
How or where can supporting documents be obtained?	—
When will supporting documents availability begin?	—
When will supporting documents availability end?	—
To whom will data be available?	—
For what type of analysis or purpose?	—
By what mechanism?	—
Any other restrictions?	—
Additional information	—

This statement was posted on November 8, 2025, at NEJM.org.