Supplementary Appendix

Supplement to: Laffin LJ, Nicholls SJ, Scott RS, et al. Durability of CRISPR-Cas9 gene editing targeting ANGPTL3 with CTX310. N Engl J Med. DOI: 10.1056/NEJMc2609825

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

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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
Shoa Clarke	Palo Alto, CA, USA

Biomarker measurement

Fasting blood samples were collected to evaluate lipid parameters. Samples were analyzed at a central laboratory on an autoanalyzer.

The initial report of remnant cholesterol changes reported calculated values[1]. Following that publication, remnant cholesterol was measured directly and is reported herein.

Plasma levels ANGPTL3 were measured utilizing an enzyme-linked immunosorbent assay (R&D Systems).

1. Laffin LJ, Nicholls SJ, Scott RS, Clifton PM, Baker J, Sarraju A, Singh S, Wang Q, Wolski K, Xu H, Nielsen J, Patel N, Duran JM, Nissen SE. Phase 1 Trial of CRISPR-Cas9 Gene Editing Targeting ANGPTL3. N Engl J Med. 2025 Nov 27;393(21):2119-2130.

Estimated lean body weight calculation

The lean body weight was estimated (eLBW) using the following formula[2]:

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.

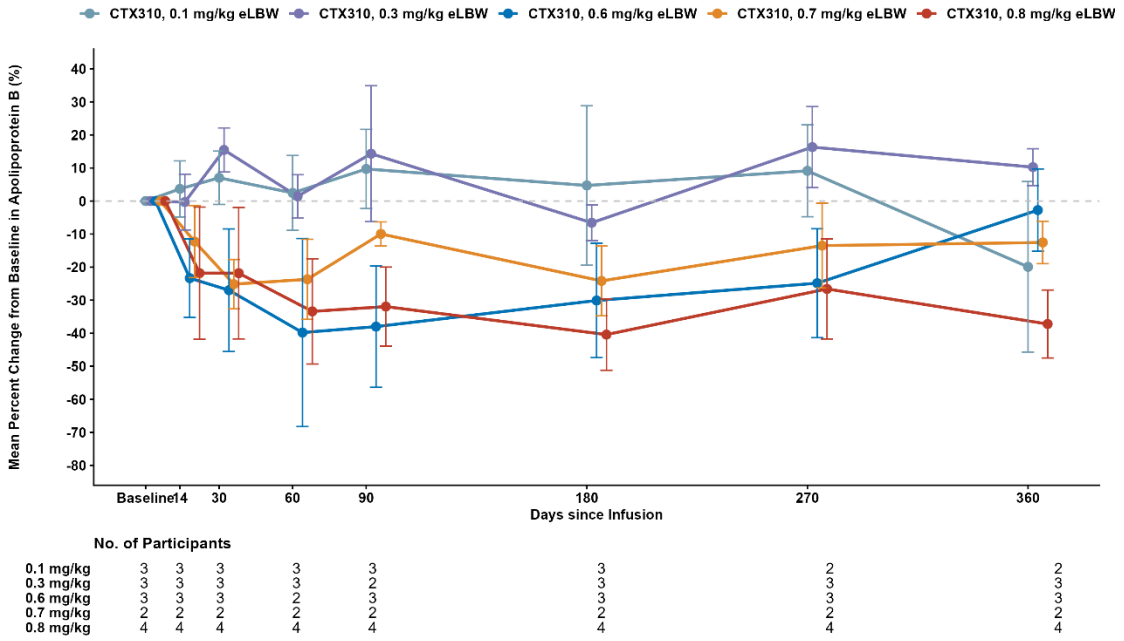
2. Janmahasatian S, Duffull SB, Ash S, Ward LC, Byrne NM, Green B. Quantification of lean bodyweight. Clin Pharmacokinet. 2005;44:1051–1065.

Study objectives and end points

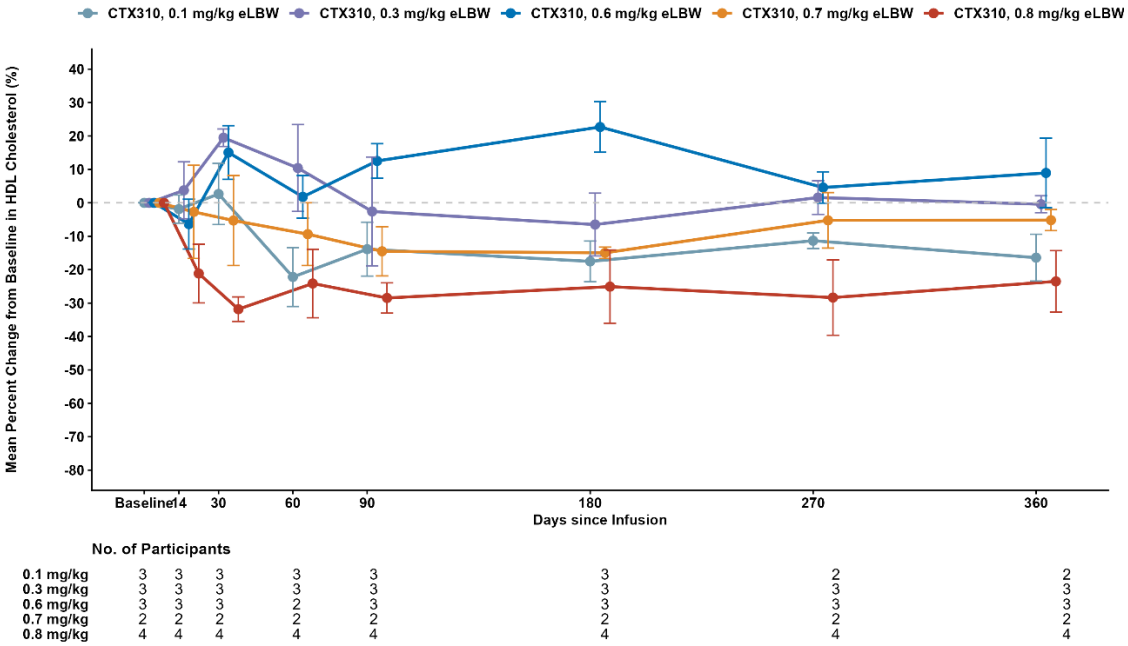
Primary Objectives	Primary End Points
• To evaluate the safety and tolerability of a single ascending dose of CTX310 in participants with refractory dyslipidemias with elevated levels of TG and/or non–HDL-C and/or ApoB and/or LDL-C, and to determine the recommended Phase 2 dose	• Incidence of dose-limiting 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

Figure S1. Changes in apolipoprotein B, HDL cholesterol, and triglyceride-rich lipoprotein cholesterol levels over time with CTX310

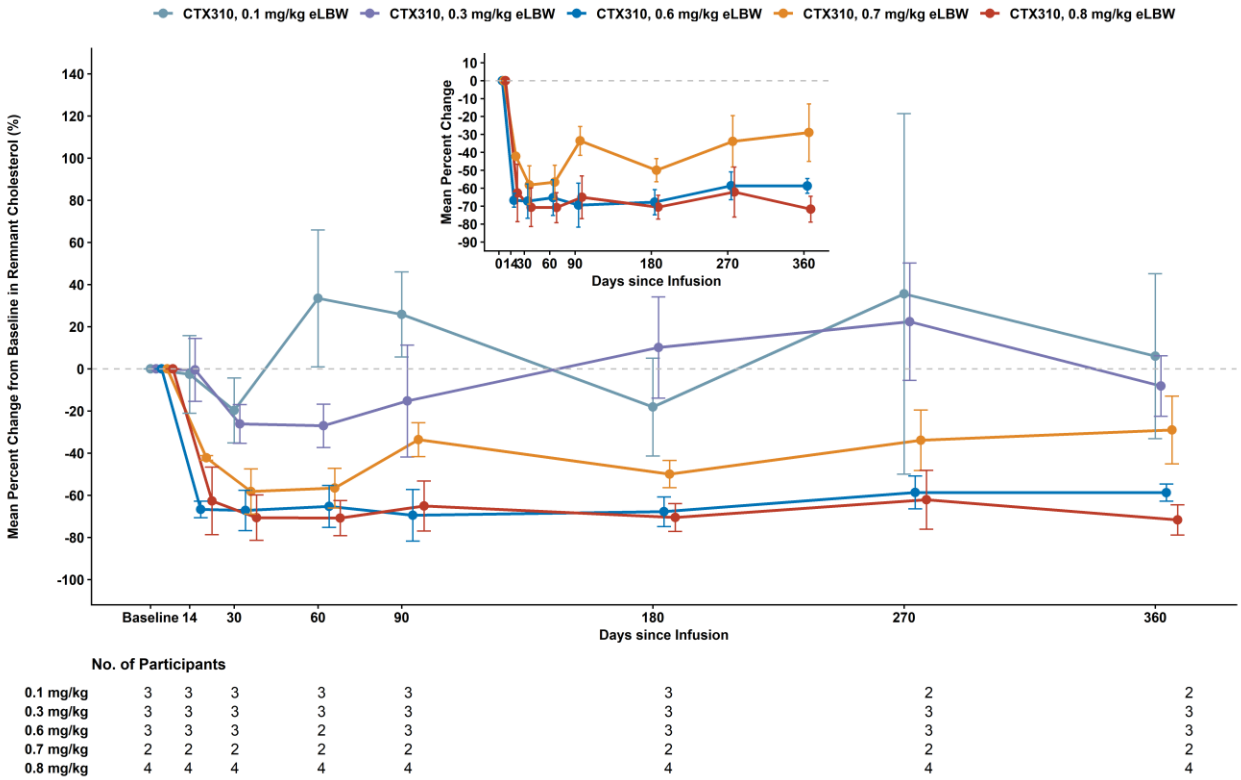
Panel A (apolipoprotein B)



Panel B (HDL cholesterol)



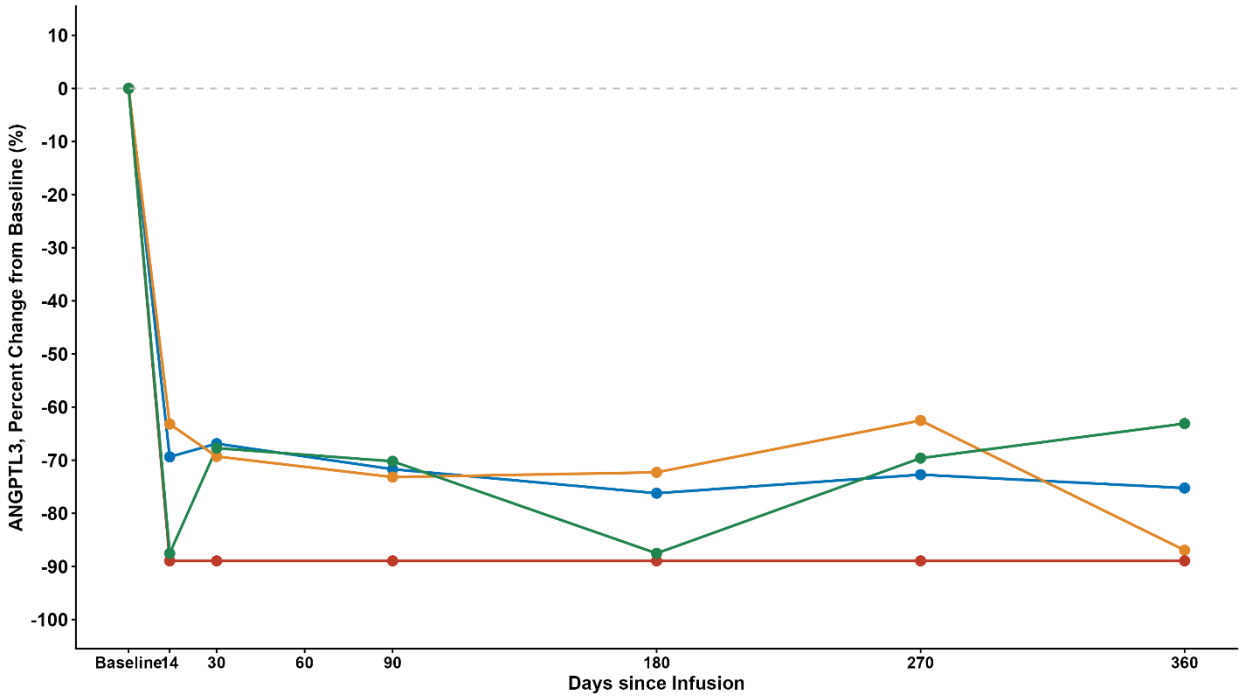
Panel C (triglyceride-rich lipoprotein cholesterol)



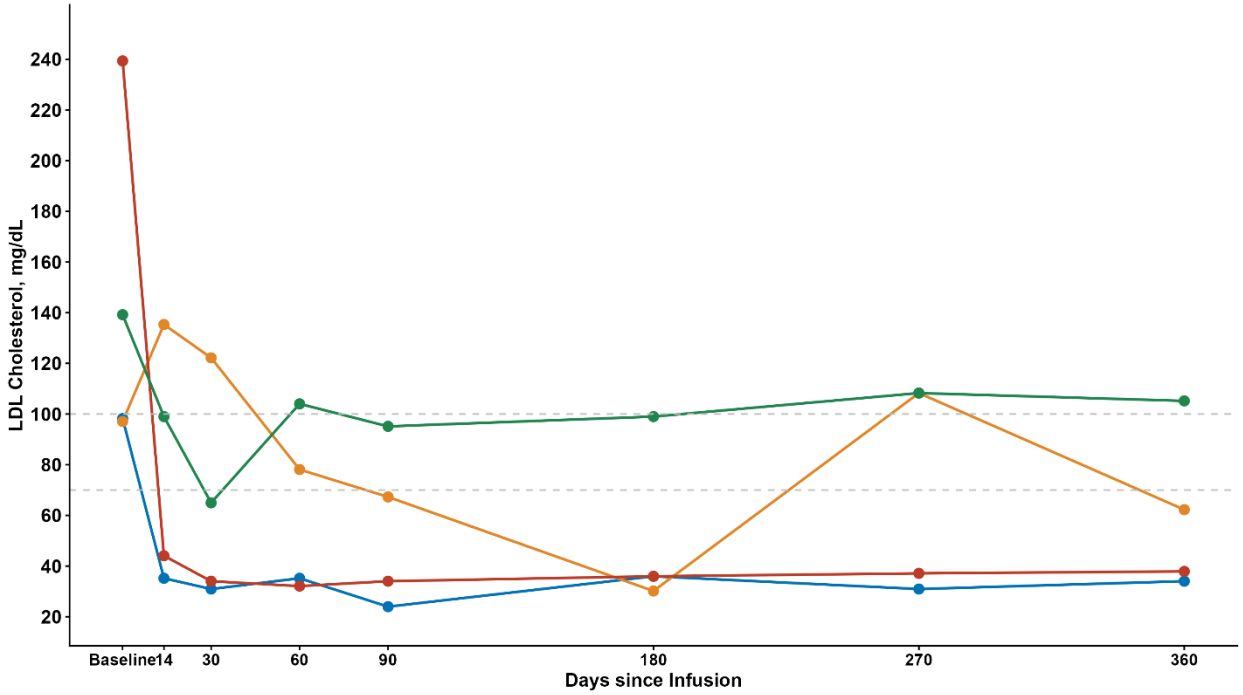
Panel A shows the mean percent change from baseline in apolipoprotein B levels by visit for each dose level. Panel B shows the mean percent change from baseline in high-density lipoprotein (HDL) cholesterol levels by visit for each dose level. Panel C shows the mean percent change from baseline in triglyceride-rich cholesterol levels by visit for each dose level. I bars indicate standard errors. The abbreviation eLBW denotes estimated lean body weight.

Figure S2. Individual participant changes in ANGPTL3, LDL cholesterol, triglycerides following treatment with 0.8 mg/kg of CTX310

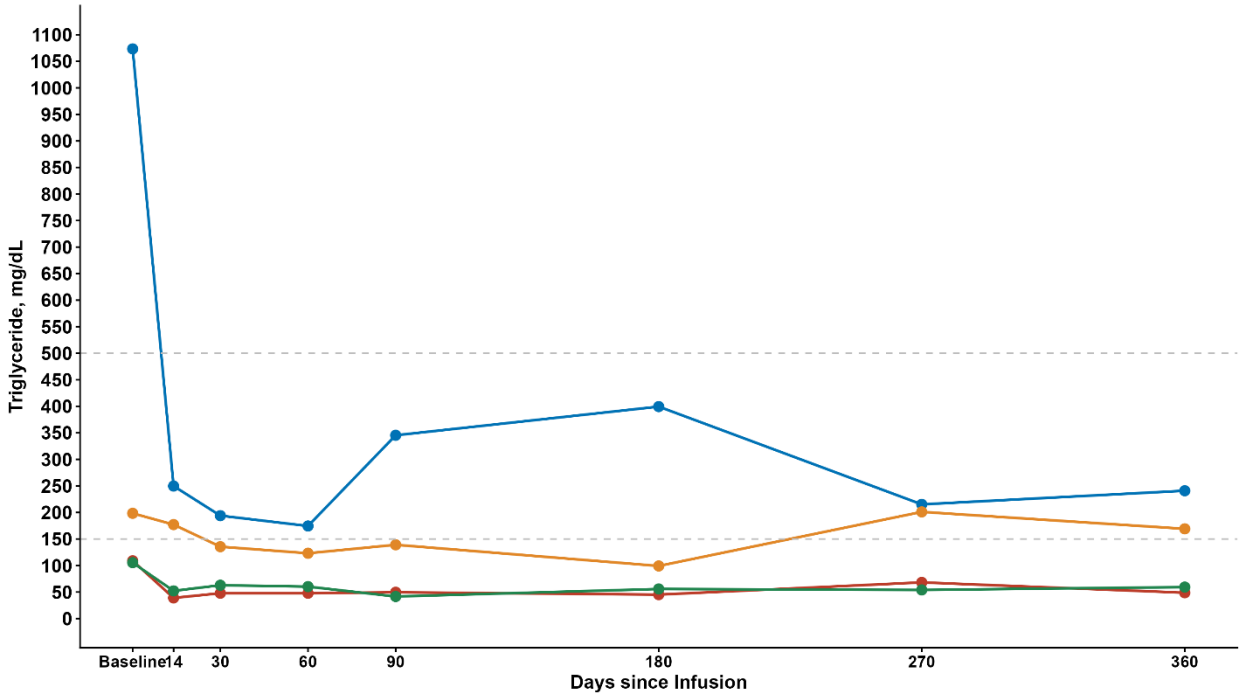
Panel A (ANGPTL3)



Panel B (LDL cholesterol)



Panel C (Triglycerides)



Panel A shows the percent change from baseline in angiopoietin-like protein 3 (ANGPTL3) levels for each individual participant in the 0.8 mg/kg cohort. Panel B shows change in low-density lipoprotein cholesterol (LDL-C) levels for each individual participant in the 0.8 mg/kg cohort. Panel C shows change in triglyceride levels for each individual participant in the 0.8 mg/kg cohort. Each color represents an individual participant. Of the four participants enrolled in the 0.8 mg/kg cohort, one was enrolled with a diagnosis of hypertriglyceridemia (blue), one with a diagnosis hypercholesterolemia (red), and two with a diagnosis of mixed dyslipidemia (green and orange).

Table S1. Adverse events through full 12-month follow-up

	CTX310 (n=15)
	no. (%)
Death*	1 (7)
Any serious adverse events[†]	1 (7)
Serious adverse events related to CTX310	0 (0.0)
Participants with 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)*
Participants with adverse event deemed by investigators as related to CTX310[¶]	7 (47)
Grade 1	3 (20)[#]
Grade 2	4 (27)^
Grade 3	0 (0.0)
Grade 4	0 (0.0)
Grade 5	0 (0.0)
Participants with an adverse event of special interest related to CTX310[¶]	4 (27)
Allergic or localized reaction	1 (7)
Infusion-related reaction[‡]	3 (20)
Elevation in AST or ALT[§]	1 (7)

*One death occurred in a participant 176 days after treatment with the 0.1 mg/kg dose. The patient's death was deemed unrelated to CTX310.
[†]Two serious adverse events were reported in a publication of early data. This included the one participant death, and a hospitalization for spinal disk herniation 7 months following treatment in a participant treated with a 0.3 mg/kg dose. Following this initial publication, it was subsequently determined that the spinal disk herniation was a pre-existing condition and did not meet criteria for a serious adverse event.

¶ Participants were counted once at the highest-grade adverse event based on Common Terminology Criteria for Adverse Events.
[&] Insomnia in 1 participant, raised white blood cell count in 1, acute kidney injury in 1, cerumen impaction in 1. These occurred in participants without a treatment-emergent adverse event related to CTX310.
[@] Muscles aches in 1 participant (no event related to CTX310 in this participant), head injury in 1 participant (same participant had grade 1 event of fatigue deemed related to CTX310), spinal disk herniation in 1 (same participant had grade 2 raised white blood cell count deemed related to CTX310), headache/toothache in 1 (same participant had grade 1 raised white blood cell count deemed related to CTX310), leg infection in 1 (no event related to CTX310 in this participant), gastroenteritis in 1 (same participant had grade 1 event of fatigue deemed related to CTX310), infusion-related reaction in 3 participants (all deemed related to CTX310, 1 participant had concomitant aminotransferase elevation).
[#] Fatigue and lightheadedness in 1 participant, fatigue in 1, raised white blood cell count in 1.
^ Infusion-related reaction in 2 participants, infusion-related reaction and aminotransferase elevation in 1, raised white blood cell count in 1.
‡ Occurred in 2 participants at 0.6 mg/kg dose and in 1 at 0.8 mg/kg dose.
[§] Increase in ALT or AST ≥3x the upper-limit of normal or ≥2x the baseline value (if baseline ALT was greater than the upper-limit of normal at time of enrollment).
ALT denotes alanine aminotransferase and AST aspartate aminotransferase.

Table S2. Effect of CTX310 on ANGPTL3 and lipid biomarkers at one year

Dose group	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 (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 at Month 12 (range) — ng/mL	110.4 (62.0 to 158.7)	103.4 (94.6 to 110.5)	59.5 (42.3 to 83.2)	28.6 (18.2 to 39.0)	32.0 (18.2 to 53.7)
Mean absolute change (range) — ng/mL	-10.9 (-26.3 to 4.4)	-59.5 (-92.0 to -37.5)	-98.3 (-107.2 to -81.2)	-106.7 (-119.8 to -93.7)	-118.6 (-146.1 to -91.9)
Mean percent change (range) — %	-13.4 (-29.7, 2.9)	-35.2 (-45.4 to -28.4)	-62.8 (-71.6 to -56.3)	-78.7 (-86.8 to -70.6)	-78.6 (-89.0 to -63.1)
LDL cholesterol					
Mean baseline (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 at Month 12 (range) — mg/dL	194.3 (80.0 to 308.6)	169.0 (30.9 to 269.5)	162.9 (139.2 to 181.4)	121.2 (71.2 to 171.3)	59.8 (34.0 to 105.2)
Mean absolute change (range) — mg/dL	-13.9 (-27.1 to -0.8)	19.1 (5.0 to 40.2)	-17.3 (-88.2 to 18.2)	-6.4 (-22.8 to 10.1)	-83.6 (-201.5 to -34.0)
Mean percent change (range) — %	-12.8 (-25.3 to -0.2)	16.1 (4.7 to 24.2)	-2.7 (-34.4 to 15.0)	-9.0 (-24.3 to 6.2)	-52.5 (-84.2 to -24.4)
Triglycerides					
Mean baseline (range) — mg/dL	141.4 (83.3 to 184.2)	464.1 (135.5, 1007.9)	248.0 (192.2 to 299.4)	148.8 (74.4 to 223.2)	371.6 (105.4 to 1073.5)
Mean at Month 12 (range) — mg/dL	220.5 (217.0 to 224.1)	387.3 (124.0 to 817.5)	121.3 (93.9 to 163.9)	174.0 (91.2 to 256.9)	129.5 (48.7 to 240.9)
Mean absolute change (range) — mg/dL	50.0 (39.9 to 60.2)	-76.8 (-190.4 to -11.5)	-126.7 (-146.1 to -98.3)	25.2 (16.8 to 33.7)	-242.0 (-832.6 to -29.2)
Mean percent change (range) — %	30.0	-12.9	-51.4	18.8	-47.8

	(21.6 to 38.4)	(-18.9 to -8.5)	(-57.9 to -45.3)	(15.1 to 22.6)	(-77.6 to -14.7)
Apolipoprotein B					
Mean baseline (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 at Month 12 (range) — mg/dL	136.5 (57.0 to 216.0)	151.0 (86.0 to 191.0)	143.0 (114.0 to 161.0)	107.5 (77.0 to 138.0)	69.5 (57.0 to 81.0)
Mean absolute change (range) — mg/dL	-18.0 (-48.0 to 12.0)	12.0 (-1.0 to 27.0)	-10.7 (-57.0 to 20.0)	-13.5 (-18.0 to -9.0)	-46.8 (-90.0 to -12.0)
Mean percent change (range) — %	-19.9 (-45.7 to 5.9)	10.3 (-0.5 to 18.1)	-2.7 (-27.0 to 14.2)	-12.5 (-18.9 to -6.1)	-37.2 (-61.2 to -12.9)
HDL cholesterol					
Mean baseline (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 at Month 12 (range) — mg/dL	36.9 (32.9 to 41.0)	29.8 (22.0 to 35.2)	41.8 (37.1 to 45.2)	35.6 (34.0 to 37.1)	47.1 (20.9 to 71.2)
Mean absolute change (range) — mg/dL	-7.2 (-10.1 to -4.3)	-0.0 (-1.2 to 1.2)	3.1 (-5.0 to 7.3)	-1.9 (-3.1 to -0.8)	-12.2 (-18.9 to 0.0)
Mean percent change (range) — %	-16.4 (-23.4 to -9.4)	-0.4 (-5.0 to 3.7)	8.9 (-11.9 to 19.4)	-5.2 (-8.3 to -2.0)	-23.5 (-44.9 to 0.0)
Non-HDL cholesterol					
Mean baseline (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 202.2)	188.7 (136.1 to 263.3)
Mean at Month 12 (range) — mg/dL	240.3 (108.3 to 372.4)	250.7 (203.4 to 297.4)	193.4 (161.3 to 215.4)	144.2 (86.2 to 202.2)	80.8 (54.1 to 116.0)
Mean absolute change (range) — mg/dL	2.9 (-27.1 to 32.9)	16.1 (3.9 to 35.2)	-40.0 (-100.9 to -8.9)	-11.4 (-22.8 to 0.0)	-107.9 (-209.2 to -39.1)
Mean percent change (range) — %	-5.2 (-20.0 to 9.7)	7.5 (1.3 to 16.3)	-14.0 (-31.9 to -4.2)	-10.5 (-20.9 to 0.0)	-52.0 (-79.4 to -25.2)
Triglyceride-rich lipoprotein cholesterol^					
Mean baseline (range) — mg/dL	67.9 (29.9 to 115.1)	95.0 (85.4 to 108.5)	96.8 (85.2 to 119.3)	50.5 (28.2 to 72.7)	62.9 (37.8 to 100.5)
Mean at Month 12 (range) — mg/dL	103.2 (39.2 to 167.1)	87.5 (59.1 to 108.0)	40.4 (28.4 to 54.5)	39.4 (15.5 to 63.3)	16.2 (7.2 to 21.2)

Mean absolute change (range) — mg/dL	16.3 (-19.4 to 52.0)	-7.6 (-26.3 to 16.8)	-56.3 (-64.8 to -46.8)	-11.1 (-12.7 to -9.4)	-46.7 (-79.3 to -21.2)
Mean percent change (range) — %	6.0 (-33.1 to 45.2)	-8.2 (-30.8 to 18.4)	-58.7 (-66.9 to -54.3)	-29.0 (-45.0 to -12.9)	-71.7 (-87.8 to -56.1)

^Directly measured. ANGPTL3 denotes angiopoietin-like 3; HDL high-density lipoprotein; LDL low-density lipoprotein. SI units: To convert LDL cholesterol, HDL cholesterol, and non-HDL cholesterol to nmol/L, multiply by 0.0259. To convert triglycerides to nmol/L multiply by 0.0113.

Table S3. Representativeness of trial population

Disease Under Investigation	**Hypertriglyceridemia**
Special Considerations related to:	
Sex and Gender	Both women and men are affected by hypertriglyceridemia, although severe hypertriglyceridemia (≥500 mg/dL) is more frequently identified in men. In pooled contemporary NHANES data, 24.2% of U.S. adults with triglycerides ≥500 mg/dL were women and 75.8% were men (Gurevitz). An earlier NHANES analysis similarly found that 75.3% of adults with severe hypertriglyceridemia were men (Christian).
Race and Ethnicity	Hypertriglyceridemia occurs across all racial and ethnic populations, although its measured prevalence varies. In an NHANES analysis of severe hypertriglyceridemia, 70.1% of affected participants were non-Hispanic White (Christian). In NHANES 2001–2012, the prevalence of elevated triglyceride concentrations (≥150 mg/dL) was consistently lower among non-Hispanic Black adults than among non-Hispanic White or Mexican American adults (Carroll). There were no restrictions to enrollment in the current trial 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 hypertriglyceridemia. Hypertriglyceridemia affects adults across the life course, with severe hypertriglyceridemia frequently identified during middle age. In an NHANES analysis, 58.5% of adults with severe hypertriglyceridemia were 40 to 59 years of age (Christian).
Overall representativeness of this trial	This first-in-human, ascending-dose study enrolled only 15 participants at six specialized sites in Australia, New Zealand, and the United Kingdom. Its age distribution and predominance of men were generally consistent with epidemiologic observations in severe hypertriglyceridemia, but women and racial and ethnic minority groups were underrepresented. Eight total participants had hypertriglyceridemia, 6 eligible for the trial due to a mixed dyslipidemia and 2 eligible for enrollment due to severe hypertriglyceridemia. Larger studies with broader geographic, demographic, and phenotypic representation will be necessary to establish the generalizability of the safety and lipid effects of CTX310.

Data cited from:

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

Christian JB, Bourgeois N, Snipes R, Lowe KA. Prevalence of severe (500 to 2,000 mg/dl) hypertriglyceridemia in United States adults. Am J Cardiol. 2011;107(6):891-897.

Carroll MD, Kit BK, Lacher DA. Trends in elevated triglyceride in adults: United States, 2001–2012. NCHS data brief, no 198. Hyattsville, MD: National Center for Health Statistics. 2015.