Supplemental Material

Table of Contents

Supplemental Methods:

STUDY POPULATION. The ACCENTUATE trial (supported by Eli Lilly and Company, ClinicalTrials.gov Identifier: NCT02227784) was a Phase 3 randomized clinical trial designed to evaluate the effects of evacetrapib on HDL cholesterol and other lipids in participants with high cholesterol and atherosclerotic cardiovascular disease (ASCVD) and/or diabetes. The inclusion criteria required participants to be over 18 years of age, diagnosed with atherosclerotic cardiovascular disease or diabetes mellitus (type 1 or type 2), have elevated LDL (>70mg/dL) or non-HDL (>100mg/dL) cholesterol, triglyceride ≤400mg/dL, and be treated with atorvastatin 40mg daily for at least 30 days prior to screening. Participants were excluded for recent stroke or acute coronary syndrome, uncontrolled hypertension, documented hyperaldosteronism, uncontrolled diabetes, malignancy, or significant liver, kidney, or cardiac disease. Following a 28-day lead-in period of atorvastatin 40mg daily, 366 eligible participants were randomized to four treatment arms (2:1:1:2): 123 assigned to evacetrapib 130mg and atorvastatin 40mg, 54 assigned to atorvastatin 40mg, 62 assigned to atorvastatin 80mg, and 127 assigned to ezetimibe 10mg and atorvastatin 40mg. Our study compared all of the subjects included in the final efficacy analysis from two of these treatment arms: atorvastatin 40mg and evacetrapib 130mg daily (n=86); and atorvastatin 40mg and placebo evacetrapib (n=40) daily.

The ILLUMINATE trial (supported by Pfizer, ClinicalTrials.gov Identifier: NCT00134264) was a Phase 3 randomized controlled trial designed to demonstrate if torcetrapib plus atorvastatin could reduce the risk for major cardiovascular disease events compared to atorvastatin alone in patients with coronary heart disease or risk equivalents. The inclusion criteria required participants to be 45 to 75 years of age and diagnosed with cardiovascular disease or type 2 diabetes 30 days to 5 years before screening. Exclusion criteria included unstable medical condition, life expectancy <5yrs, LDL cholesterol <100mg/dL

in patients not receiving lipid-altering medication, cardiovascular event or hypertension during run in, or if the LDL cholesterol target was not reached by the end of run-in. During a 4 to 10 week run-in period, atorvastatin was administered at four levels determined by the minimum required dose to achieve the LDL cholesterol target of <100mg/dL. Those who met this target were randomized to continue their regimen of atorvastatin with the addition of either torcetrapib 60mg daily (n=7526) or placebo-torcetrapib (n=7528). From these participants, our study randomly selected 20 men and 20 women from the atorvastatin 10mg + torcetrapib 60mg daily dose group and an additional 40 participants from the atorvastatin 10mg group + placebo torcetrapib who were matched on sex, race, and age (within 1 year).

LABORATORY ANALYSIS. Stored plasma samples from the ACCENTUATE trial of evacetrapib and the ILLUMINATE trial of torcetrapib collected at baseline and after three months were shipped on dry ice by overnight courier to the Harvard T.H. Chan School of Public Health and stored at -80°C pending analysis. Samples were thawed at room temperature and gently swirled to ensure homogeneity. Modified sandwich ELISAs to quantify the concentrations of apoA1 in the 17 HDL subspecies defined by selected proteins were performed as described in detail previously. Briefly, samples were diluted in phosphate-buffered saline and loaded into 16 prepared 96-well microplates, each one coated with a different antibody corresponding to the 16 subspecies-defining proteins. Following overnight incubation at 4°C, the unbound fraction depleted of the HDL subspecies that contains the defining protein was removed. Of all the unbound fractions produced, only the unbound fraction from the anti-apoA2 plate was measured. This was recorded as the apoA1 concentration of “HDL that lacks apoA2”. This fraction was measured because unlike the other protein-defined HDL subspecies, HDL that contains apoA2 makes up the majority fraction of HDL (~80%) while HDL that lacks apoA2 is the minority fraction. The plates were washed gently three times with PBS, then loaded with tween-containing diluent and incubated for 1hr at 37°C to dissociate the lipoprotein complexes bound to the plate. The dissociated sample was transferred

to a prepared 96-well microplate coated with anti-apoA1 antibody, one per HDL subspecies plus one more for the HDL lacking apoA2. The 17 plates were incubated for 1hr at 37°C, then washed three times. Biotinylated anti-apoA1 was loaded, plates were incubated and washed, streptavidin was added, plates were incubated and washed, and finally o-phenylenediamine substrate was added and incubated in the dark (all incubations at 37°C for 1hr). To minimize batch effects, baseline and 3-month samples were paired side-by side in the same ELISA plate in random order and CETP-treated and untreated samples were alternated with equal numbers on each ELISA plate. The lab was blinded to time point and treatment status. In all cases, ELISAs were judged on the quality of the calibration curve, the correlation of obtained and expected values of the control samples (required $r>0.7$), and the coefficients of variation for the unknown samples. Extreme outliers could be removed from calibration curves, but curves had to be produced from at least 4 of the 7 calibration curve points and show a fit of $r^2>0.95$. The average coefficient of variation (%CV) for all replicate samples could not exceed 15% for a plate to be accepted. Individual replicates whose %CV exceeded 20% were repeated.

Supplemental Table S1. Effects of evacetrapib. Median apoA1 concentration (mg/dL) of total plasma and of protein-defined HDL subspecies at baseline and after placebo or evacetrapib treatment with interquartile range, median change from baseline, and percent change from baseline. IQR – interquartile range. *FDR-adjusted p-value <0.05 for the comparison of the effect of placebo to evacetrapib.

	pre-treatment		post-treatment		median	median
	median	IQR	median	IQR	change	% change
evacetrapib						
total plasma apoA1	131	105-166	212	185-268	84 *	65 *
HDL that lacks apoA2	36	28-50	62	45-91	24 *	72 *
HDL that contains apoA2	95	76-123	149	128-181	55 *	59 *
HDL that contains alpha-1-antitrypsin	9.58	5.36-17.37	12.46	8.15-22.09	2.49 *	48 *
HDL that contains alpha-2-macroglobulin	2.06	1.24-3.49	2.65	1.98-3.83	0.50 *	27 *
HDL that contains apoA4	2.84	2.15-4.54	5.14	4.14-6.67	2.09 *	76 *
HDL that contains apoC1	11.92	7.06-16.43	21.20	12.98-28.31	7.51 *	79 *
HDL that contains apoC2	1.28	0.87-2.03	1.89	1.35-2.49	0.54 *	46 *
HDL that contains apoC3	5.75	3.79-9.74	11.77	7.58-17.52	5.16 *	93 *
HDL that contains complement C3	2.99	1.79-6.14	4.33	2.50-6.42	0.87 *	27 *
HDL that contains ceruloplasmin	1.33	0.90-2.42	1.76	1.19-2.64	0.32 *	27 *
HDL that contains apoE	7.98	5.22-13.18	16.73	10.20-25.61	7.97 *	91 *
HDL that contains fibrinogen	2.37	1.49-3.92	3.29	2.62-4.54	0.88 *	38 *
HDL that contains haptoglobin	1.81	1.17-2.83	2.10	1.48-2.93	0.31 *	17 *
HDL that contains apoJ	1.73	1.16-2.67	2.31	1.78-3.20	0.61 *	40 *
HDL that contains apoL1	3.28	2.33-4.75	3.57	3.10-4.43	0.36	10
HDL that contains plasminogen	3.11	1.93-5.77	4.36	3.28-5.64	0.70 *	30 *
HDL that contains paraoxonase-1	8.51	6.43-13.71	10.89	8.63-14.17	2.20 *	27 *
placebo						
total plasma apoA1	139	98-169	144	116-183	5	4
HDL that lacks apoA2	40	26-56	42	31-53	-1	-4
HDL that contains apoA2	103	59-123	100	76-132	13	16
HDL that contains alpha-1-antitrypsin	8.60	5.72-16.46	8.42	6.26-12.10	-1.89	-21
HDL that contains alpha-2-macroglobulin	1.86	1.47-2.96	1.94	1.49-2.83	-0.08	-5
HDL that contains apoA4	2.83	2.25-3.83	3.06	2.18-3.91	0.11	5
HDL that contains apoC1	12.18	8.19-18.23	12.80	7.48-18.87	0.77	8
HDL that contains apoC2	1.38	0.99-1.75	1.24	0.91-1.77	-0.07	-8
HDL that contains apoC3	6.52	4.17-7.80	6.06	4.42-8.11	-0.25	-6
HDL that contains complement C3	2.66	1.83-5.34	2.92	1.59-4.88	-0.18	-3
HDL that contains ceruloplasmin	1.25	0.91-1.78	1.21	0.91-1.56	-0.16	-14
HDL that contains apoE	7.69	5.84-11.95	8.11	4.99-13.32	0.55	6
HDL that contains fibrinogen	2.59	1.74-3.41	2.57	1.71-3.52	-0.15	-5
HDL that contains haptoglobin	1.83	0.92-2.85	1.55	1.04-2.42	-0.26	-16
HDL that contains apoJ	1.74	1.31-2.13	1.56	1.25-2.00	-0.12	-8
HDL that contains apoL1	3.02	2.36-3.82	3.02	2.38-3.75	-0.06	-2
HDL that contains plasminogen	3.02	2.23-4.21	2.62	1.80-3.50	-0.40	-13
HDL that contains paraoxonase-1	8.28	6.03-11.74	8.13	6.87-10.72	0.03	0

Supplemental Table S2. Effects of torcetrapib. Median apoA1 concentration (mg/dL) of total plasma and of protein-defined HDL subspecies at baseline and after placebo or torcetrapib treatment with interquartile range, median change from baseline, and percent change from baseline. IQR – interquartile range. *FDR-adjusted p-value <0.05 for the comparison of the effect of placebo to torcetrapib.

	pre-treatment		post-treatment		median	median
	median	IQR	median	IQR	change	% change
torcetrapib						
total plasma apoA1	143	124-183	196	159-225	37 *	27 *
HDL that lacks apoA2	29	23-48	47	33-58	10 *	36 *
HDL that contains: apoA2	114	100-143	149	121-165	29 *	28 *
HDL that contains: alpha-1-antitrypsin	10.47	6.09-23.61	15.33	8.31-28.21	1.61	20
HDL that contains: alpha-2-macroglobulin	2.19	1.48-3.32	3.13	2.09-4.48	0.74	46
HDL that contains: apoA4	3.55	2.61-4.07	4.68	3.48-5.36	0.73	23
HDL that contains: apoC1	13.08	9.60-16.11	19.12	13.68-23.32	4.75 *	45 *
HDL that contains: apoC2	2.03	1.25-2.81	2.45	1.57-3.07	0.40	26
HDL that contains: apoC3	6.70	4.57-7.91	9.24	7.87-13.70	2.66 *	49 *
HDL that contains: complement C3	3.20	2.41-4.75	4.00	3.06-5.75	0.70	26
HDL that contains: ceruloplasmin	1.24	0.95-1.87	1.70	1.08-2.60	0.39	32
HDL that contains: apoE	10.21	6.15-13.28	14.08	10.20-22.20	3.61 *	42 *
HDL that contains: fibrinogen	3.09	2.09-3.76	3.44	2.31-4.28	0.54	17
HDL that contains: haptoglobin	1.72	1.33-2.44	2.10	1.68-2.67	0.26	13
HDL that contains: apoJ	1.43	1.18-2.03	1.91	1.63-2.46	0.43	31 *
HDL that contains: apoL1	2.71	2.38-3.47	3.21	2.77-3.75	0.27	9
HDL that contains: plasminogen	3.72	2.52-5.26	4.14	3.60-5.51	0.69	27
HDL that contains: paraoxonase-1	9.39	7.25-11.88	9.81	8.10-14.62	1.24	17
placebo						
total plasma apoA1	142	118-164	139	124-157	-5	-3
HDL that lacks apoA2	32	25-39	31	26-38	-1	-2
HDL that contains: apoA2	108	91-125	108	96-122	-1	-0.5
HDL that contains: alpha-1-antitrypsin	9.14	5.75-13.57	12.80	7.54-19.35	1.43	15
HDL that contains: alpha-2-macroglobulin	2.21	1.88-3.53	2.78	1.70-3.63	0.00	2
HDL that contains: apoA4	3.01	2.28-4.43	3.61	2.62-4.87	0.34	13
HDL that contains: apoC1	13.35	10.37-15.53	12.35	9.96-15.59	0.57	5
HDL that contains: apoC2	1.82	1.48-2.30	2.30	1.38-2.59	0.22	12
HDL that contains: apoC3	6.15	4.78-8.67	6.19	4.81-8.57	-0.10	-2
HDL that contains: complement C3	3.37	2.73-4.30	3.54	2.51-6.05	0.19	7
HDL that contains: ceruloplasmin	1.53	0.93-1.96	1.90	1.00-2.71	0.32	26
HDL that contains: apoE	8.22	5.70-13.53	8.05	5.82-14.80	0.26	2
HDL that contains: fibrinogen	2.37	1.88-3.55	3.22	2.08-3.65	0.28	12
HDL that contains: haptoglobin	1.72	1.05-2.18	1.58	1.17-2.57	0.04	5
HDL that contains: apoJ	1.50	1.19-1.77	1.49	1.08-2.24	-0.02	-1
HDL that contains: apoL1	2.79	2.50-3.42	3.16	2.71-3.71	0.20	7
HDL that contains: plasminogen	3.33	2.22-4.85	3.87	2.90-5.30	0.32	12
HDL that contains: paraoxonase-1	8.88	7.14-13.06	9.87	7.39-14.85	-0.06	-1

Major Resources Table

In order to allow validation and replication of experiments, all essential research materials listed in the Methods should be included in the Major Resources Table below. Authors are encouraged to use public repositories for protocols, data, code, and other materials and provide persistent identifiers and/or links to repositories when available. Authors may add or delete rows as needed.

Animals (in vivo studies): not applicable

Genetically Modified Animals: not applicable

Antibodies

Protein	Coating Antibody	Detection Antibody
ApoA-I	Goat Polyclonal Anti-Human ApoA-I, Academy Biomedical (#11A-G2b, 5 ug/mL)	Goat Polyclonal Anti-Human ApoA-I Biotin conjugate, Academy Biomedical (#11B-G2b, 1 ug/mL)
alpha-1-antitrypsin	Rabbit Polyclonal Anti-Human A1AT, NOVUS (#NBP1-78098, 2 ug/mL)	Rabbit Polyclonal Anti-Human A1AT, NOVUS (HRP conjugate prepared in lab) (#NBP1-78098, 1 ug/mL)
alpha-2-macroglobulin	Goat Polyclonal Anti-Human A2M, Thermo Fisher Scientific (#PA1-74010, 2 ug/mL)	Goat Polyclonal Anti-Human A2M Biotin conjugate, Thermo Fisher Scientific (#PA1-28857, 1 ug/mL)
ApoA-II	Goat Polyclonal Anti-Human ApoA-II, Academy Biomedical (#12A-G1b, 5 ug/mL)	Goat Polyclonal Anti-Human ApoA-II HRP conjugate, Academy Biomedical (#12H-G2b, 1 ug/mL)
apoA-IV	Rabbit Polyclonal Anti-Human apoA-IV Proteintech (#17996-1-AP, 2 ug/mL)	Rabbit Polyclonal Anti-Human apoA-IV Millipore (#SPR687CA, 1 ug/mL)
ApoC-I	Goat Polyclonal Anti-Human ApoC-I, Academy Biomedical (#31A-G1b, 5 ug/mL)	Goat Polyclonal Anti-Human ApoC-I HRP conjugate, Academy Biomedical (#31H-G1b, 1 ug/mL)
ApoC-II	Goat Polyclonal Anti-Human ApoC-II, Academy Biomedical (#32A-G1b, 2 ug/mL)	Goat Polyclonal Anti-Human ApoC-II HRP conjugate, Academy Biomedical (#32H-G4b, 1 ug/mL)
ApoC-III	Goat Polyclonal Anti-Human ApoC-III, Academy Biomedical (#33A-G1b, 5 ug/mL)	Goat Polyclonal Anti-Human ApoC-III HRP conjugate, Academy Biomedical (#33H-G2b, 1 ug/mL)
ApoE	Goat Polyclonal Anti-Human ApoE, Academy Biomedical (#50H-G1b, 10 µg/mL)	Goat Polyclonal Anti-Human ApoE HRP conjugate, Academy Biomedical (#50S-G1b, 1 ug/mL)

ApoJ	Mouse Monoclonal Anti-Human ApoJ, R&D Systems (#MAB29372, 3 ug/mL)	Mouse Monoclonal Anti-Human ApoJ Biotin conjugate, R&D Systems (#BAM29373, 1 ug/mL)
apoL-I	Rabbit Polyclonal Anti-Human apoL-I Proteintech (#11486-2-AP, 5 ug/mL)	Rabbit Polyclonal Anti-Human apoL-I (Biotin conjugate prepared in lab) Proteintech (#11486-2-AP, 1 ug/mL)
Plasminogen	Goat Polyclonal Anti-Human Plasminogen, Academy Biomedical (#PG60A-G1b, 2 ug/mL)	Goat Polyclonal Anti-Human Plasminogen HRP conjugate, Academy Biomedical (#PG60H-G1a, 1 ug/mL)
fibrinogen (α, β, γ-chains)	Chicken Polyclonal Anti-Human Fibrinogen Thermo Fisher Scientific (#PA1-9526, 1 ug/mL)	Chicken Polyclonal Anti-Human Fibrinogen (Biotin conjugate prepared in lab) Thermo Fisher Scientific (#PA1-9526, 1 ug/mL)
complement C3	Goat Polyclonal Anti-Human Complement C3 Thermo Fisher Scientific (#PA1-29715, 5 ug/mL)	Goat Polyclonal Anti-Human Complement C3 (Biotin conjugate prepared in lab) Thermo Fisher Scientific (#PA1-29715, 1 ug/mL)
ceruloplasmin	Rabbit Polyclonal Anti-Human Ceruloplasmin LifeSpan BioSciences (#LS-C147360, 2 ug/mL)	Rabbit Polyclonal Anti-Human Ceruloplasmin (HRP conjugate made in lab) LifeSpan BioSciences (#LS-C147360, 1 ug/mL)
haptoglobin	Goat Polyclonal Anti-Human Haptoglobin LifeSpan BioSciences (#LS-B13232, 2 ug/mL)	Goat Polyclonal Anti-Human Haptoglobin (HRP conjugate made in lab) LifeSpan BioSciences (#LS-B13232, 1 ug/mL)
paraoxonase-1	Goat Polyclonal Anti-Human PON-1 LifeSpan BioSciences (#LS-C188035, 5 ug/mL)	Goat Polyclonal Anti-Human PON-1 (Biotin conjugate prepared in lab) LifeSpan BioSciences (#LS-C188035, 1 ug/mL)

DNA/cDNA Clones: not applicable

Cultured Cells: not applicable

Data & Code Availability

Description	Source / Repository	Persistent ID / URL
available from the corresponding author upon reasonable request		

Other: not applicable