Novel Therapeutic Approaches to Elevate HDL-C and Modulate HDL Functionality

Daniel J. Rader, MD
Perelman School of Medicine
University of Pennsylvania
Philadelphia, PA
Novel HDL Therapeutics

- Elevate plasma HDL-C
- Enhance cholesterol efflux and reverse cholesterol transport
Novel HDL Therapeutics

- Elevate plasma HDL-C
  - CETP inhibition
CETP as a Therapeutic Target
Cholesteryl Ester Transfer Protein

CETP is a 74 kDa plasma protein that circulates in plasma mainly associated with HDL.
It is a member of the LPS-binding and lipid transport protein family.
It is expressed mainly in liver and adipose tissue.
CETP exchanges neutral lipids (cholesteryl esters and triglycerides) among lipoprotein particles.
CETP has a net effect of transferring CE out of HDL in exchange for TG.

Mechanism of action of CETP

Two hypotheses have been proposed for the mechanism by which CETP transfers neutral lipids between plasma lipoproteins.

(i) a shuttle mechanism that involves CETP collecting cholesteryl esters from one lipoprotein and delivering them through the aqueous phase to another lipoprotein.

(ii) a tunnel mechanism in which CETP bridges two lipoproteins to form a ternary complex, with lipids flowing from the donor to acceptor lipoprotein through the CETP molecule.

Barter et al; Biochem J. 1982; 208:1.
Ihm et al. J. Lipid Res.1982;23:1328.
Tall. J. Lipid Res.1993;34:1255.
Shuttle Mechanism

HDL

CETP

CE
TG

CE
TG

CETP

TRL

CE
TG

CE
TG

CE
TG

CETP

CETP

CE
TG

CE
TG

CE
TG

CETP

LDL

CE
TG

CE
TG


Barter et al; Biochem J. 1982; 208:1.
Tall. J. Lipid Res.1993; 34:1255.
Tunnel Mechanism

HDL

CETP

HDL

CE

VLDL/LDL

Binary complex

CE

CETP

Tunnel complex

CE

CETP

Tunnel

CE

CE

CE

VLDL/LDL

HDL

Ihm et al. J. Lipid Res. 1982; 23, 1328.
Role of CETP in the Remodelling of HDL

Evidence that Activity of CETP Impacts on Plasma Lipoprotein Concentrations

The first genetic deficiency of CETP was identified in Japan in 1989 in patients with markedly elevated HDL-C.

57% of Japanese subjects with levels of HDL-C > 100 mg/dL have mutations of the CETP gene.

37% of Japanese subjects with levels of HDL-C between 75-100 mg/dL have mutations of the CETP gene.

CETP mutations have also been identified outside Japan in association with elevated HDL.

Common variants at the CETP locus are strongly associated with variation in HDL-C levels.

Relationship of CETP and Atherosclerosis
CETP and Atherosclerosis
Human epidemiology

Honolulu Heart Study 7-year prospective data

There was no statistically significant relation between heterozygous mutations of CETP and CHD or stroke. However, it was concluded that a deficiency of CETP is athero-protective, so long as it is accompanied by an HDL-C level > 60 mg/dL.

CETP Polymorphisms and Cardiovascular Risk in Humans

- A meta-analysis has been conducted of studies investigating relationships between CETP polymorphisms and cardiovascular disease in humans.

- 46 studies had data on 27,196 coronary cases and 55,338 controls.

- Those polymorphisms that were associated with lower CETP mass and lower CETP activity had higher levels of HDL-C and a significantly reduced coronary risk.

A prospective cohort of 18,245 initially healthy American women.

Polymorphisms near or in the CETP gene were associated with both HDL cholesterol concentration and risk of myocardial infarction.

It was concluded that decreased CETP activity is associated with an increased level of HDL-C and a decreased risk of myocardial infarction.
CETP and Atherosclerosis in Rodents

Rodents naturally deficient in CETP

Rodents naturally resistant to development of atherosclerosis

Expression of CETP in transgenic mice and rats increases atherosclerosis in most (but not all) models

CETP and Atherosclerosis in Rabbits

Rabbits have high level of activity of CETP

Rabbits naturally highly susceptible to the development of atherosclerosis

Inhibition of CETP in rabbits decreases atherosclerosis in all models, including genetic manipulation to inhibit CETP, use of an anti-CETP vaccine or by administration of small molecule CETP inhibitors (including dalcetrapib and torcetrapib)

Rittershaus et al. ATVB. 2000;20:2106.
CETP inhibition in Humans
Effect of CETP Inhibition on Plasma Cholesterol Transport

Liver

- CE
- SR-B1
- LDL-R

Bile

HDL

Extrahepatic Tissues (including the artery wall)

Free Cholesterol

CETP
CETP Inhibitors

Torcetrapib

Anacetrapib

Dalcetrapib

Evacetrapib

## Lipid efficacy of CETP inhibitors (% change from baseline)

<table>
<thead>
<tr>
<th>CETP inhibitor</th>
<th>Dose (mg/d)</th>
<th>HDL-C (%)</th>
<th>LDL-C (%)</th>
<th>TG (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torcetrapib</td>
<td>60</td>
<td>61</td>
<td>-24</td>
<td>-9</td>
</tr>
</tbody>
</table>

Adapted from Cannon C, JAMA 306:2154; 2011
Torcetrapib was an effective CETP inhibitor that was shown in humans to increase the concentration of HDL-C by more than 60% while reducing the concentration of LDL-C by more than 20% over and above the changes achieved by using high doses of statins.

Torcetrapib was the first CETP inhibitor to be tested in human imaging trials and in a large cardiovascular clinical end point trial

Coronary IVUS Trial - ILLUSTRATE

• 910 patients with angiographic coronary artery disease treated with torcetrapib 60 mg or placebo in addition to background atorvastatin for 24 months
• Change in coronary atheroma burden was determined in matched arterial segments by intravascular ultrasound
• Relationship between changes in biochemical parameters and plaque burden with treatment was determined

Nissen et al. NEJM. 2007;356:1304.
ILLUSTRATE: Primary Efficacy Parameter

Change in Percent Atheroma Volume

Nissen et al. NEJM. 2007;356:1304.
ILLUSTRATE: Secondary Efficacy Parameters

Change in Normalized Atheroma Volume (mm$^3$)

-6.3

Change in 10 mm Most Diseased Segment (mm$^3$)

-3.3

Nissen et al. NEJM. 2007;356:1304.
Atheroma Regression at Highest HDL-C Levels with Torcetrapib in the ILLUSTRATE trial

LS Mean Change Percent Atheroma Volume

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Achieved HDL Cholesterol (mg/dL)</th>
<th>LS Mean Change Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1</td>
<td>(&lt;56)</td>
<td>-0.75</td>
</tr>
<tr>
<td>Q2</td>
<td>(56-69)</td>
<td>-0.50</td>
</tr>
<tr>
<td>Q3</td>
<td>(69.5-86)</td>
<td>-0.25</td>
</tr>
<tr>
<td>Q4</td>
<td>(&gt;86)</td>
<td>0.00</td>
</tr>
</tbody>
</table>

P=0.004 for trend

ILLUSTRATE Conclusions

• Torcetrapib 60 mg in combination with atorvastatin increased HDL-C by 61% and lowered LDL-C by 20%, compared with atorvastatin monotherapy.

• However, torcetrapib also increased systolic blood pressure by an average of 4.6 mmHg.

• Torcetrapib-atorvastatin did not reduce the progression of coronary atherosclerosis for the primary efficacy parameter, compared with atorvastatin alone.

• More regression was observed with greater torcetrapib-induced elevations of HDL-C.
Carotid IMT trials: RADIANCE 1 and 2

- **B-mode ultrasound every 6 months**
  - Atorvastatin only run-in
    - Target: LDL-C to goal
  - Torcetrapib/atorvastatin
  - Atorvastatin

- **24-month double-blind treatment**

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Clinical Sites</th>
<th>Subject Population</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>RADIANCE 1</td>
<td>8 countries (US, CAN, FRA, ITA, NL, FIN, CZ, S.AFR)</td>
<td>HeFH; eligible for statin treatment</td>
<td>904</td>
</tr>
<tr>
<td>RADIANCE 2</td>
<td></td>
<td>Mixed hyperlipidemia; eligible for statin treatment</td>
<td>752</td>
</tr>
</tbody>
</table>

**RADIANCE 1 Lipids**

**HDL-C (mg/dL)**

- **T/A**: torcetrapib plus atorvastatin
- **A**: atorvastatin alone

**LDL-C (mg/dL)**

- **T/A**: torcetrapib plus atorvastatin
- **A**: atorvastatin alone

RADIANCE 1 Imaging primary Endpoint
Maximum Carotid Intima-Media Thickness (mm)

Average Over 12 Carotid Segments Combined

Means +/- SD

Months of Treatment

RADIANCE 1
Systolic Blood Pressure (mmHg)

RADIANCE 1 Conclusions

- In heterozygous FH, T/A, when compared to A alone
  - Did not result in regression of atherosclerosis as assessed by a combined measure of carotid arterial wall thickness
  - Caused progression of disease in the common carotid segment
- These effects occurred despite a 52% increase in HDL-C and a 21% decrease in LDL-C

Kastelein et al. NEJM, 2007; 356:16
RADIANCE 2 Lipids

HDL-C (mg/dL)  LDL-C (mg/dL)

Months of treatment  Months of treatment

T/A: torcetrapib plus atorvastatin  A: atorvastatin alone

RADIANCE 2 Imaging Primary Endpoint
Maximum Carotid Intima-Media Thickness (mm)
Average Over 12 Carotid Segments Combined

Means +/- SD

RADIANCE 2
Systolic Blood Pressure

In mixed dyslipidemia, torcetrapib plus atorvastatin, when compared to atorvastatin alone did not result in regression of atherosclerosis as measured by carotid arterial wall thickness. These effects occurred despite a 63% increase in HDL-C and an 18% decrease in LDL-C.

ILLUMINATE: Investigation of Lipid Level Management to Understand its Impact in Atherosclerotic Events

15,067 patients
- Men and women
- Aged 45-75 years
- 250 sites in 7 countries
- CHD or risk equivalent, any HDL-C level, statin eligible

Primary End Point
- Composite of fatal CHD, nonfatal MI, stroke (fatal and non-fatal and unstable angina requiring hospitalization)

Planned 4.5-year follow-up or 1820 events whichever is later

Torcetrapib 60 mg + titrated atorvastatin dose

Titrated atorvastatin dose

On Trial Lipid Levels By Study Month

Atorvastatin only Group (Post Run-In)

ILLUMINATE: On Trial Lipid Levels

Torcetrapib/ Atorvastatin Group (Post Run-In)

<table>
<thead>
<tr>
<th>Study Month</th>
<th>Lipids (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>127</td>
</tr>
<tr>
<td>1</td>
<td>115</td>
</tr>
<tr>
<td>3</td>
<td>112</td>
</tr>
<tr>
<td>6</td>
<td>112</td>
</tr>
<tr>
<td>12</td>
<td>112</td>
</tr>
</tbody>
</table>

TG
-9% (-27,+13)*

HDL-C
+72.1% (34.7) †

LDL-C
-24.9% (28.5) †

ILLUMINATE: Primary Endpoint: Time to First MCVE*: Kaplan-Meier Plot

Hazard Ratio 1.25
P=0.001

Event Free (%)

Atorvastatin (A) events = 373
Torcetrapib/Atorvastatin (T/A) events = 464

Days from Randomization

*Major cardiovascular event: CHD death, non-fatal MI, stroke or hospitalization for unstable angina

ILLUMINATE: Secondary Endpoint
Time to Death: Kaplan-Meier Plot

Hazard Ratio 1.58
P=0.006

## Causes of Death

<table>
<thead>
<tr>
<th>Cause</th>
<th>Atorvastatin (n=59)</th>
<th>Torcetrapib/Atorvastatin (n=93)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any cardiovascular death</td>
<td>35</td>
<td>49</td>
</tr>
<tr>
<td>Sudden cardiac death</td>
<td>25</td>
<td>26</td>
</tr>
<tr>
<td>Fatal MI - not procedure related</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>Fatal stroke</td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Other cardiac death</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Fatal heart failure</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Other vascular death/procedure related MI</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Any non-cardiovascular</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Cancer</td>
<td>14</td>
<td>24</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Other non-cardiovascular</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Trauma/suicide/homicide</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Reason unknown</td>
<td>4</td>
<td>4</td>
</tr>
</tbody>
</table>

What was the Reason for the Adverse Outcome with Torcetrapib in the ILLUMINATE Trial?

Possible explanations

• Inhibiting CETP is pro-atherogenic

• Inhibiting CETP generates dysfunctional HDL

• Torcetrapib had an adverse off-target pharmacology unrelated to CETP
What was the Reason for the Adverse Outcome with Torcetrapib in the ILLUMINATE Trial?

Possible explanations

• Inhibiting CETP is pro-atherogenic

• Inhibiting CETP generates dysfunctional HDL

• Torcetrapib had an adverse off-target pharmacology unrelated to CETP
Off-target Pharmacological Effects of Torcetrapib

In patients receiving torcetrapib in the ILLUSTRATE, RADIANCE 1 & 2 and ILLUMINATE studies there was a significant:

- Increase in blood pressure
- Decrease in serum potassium
- Increase in serum bicarbonate
- Increase in serum sodium
- Increase in serum aldosterone

Nissen et al. NEJM. 2007;356:1304.
ILLUMINATE: On-Trial Blood Pressure By Study Month

Atorvastatin Group

<table>
<thead>
<tr>
<th>Study Month</th>
<th>Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>123.0</td>
</tr>
<tr>
<td>8</td>
<td>123.9</td>
</tr>
</tbody>
</table>

Torcetrapib plus Atorvastatin Group

<table>
<thead>
<tr>
<th>Study Month</th>
<th>Blood Pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>128.2*</td>
</tr>
</tbody>
</table>

*p<0.001 vs atorvastatin at month 12

Off-target Pharmacological Effects of Torcetrapib Unrelated to CETP Inhibition

Torcetrapib increases blood pressure in rats, a species that does not have CETP.

Analogues of torcetrapib that do not inhibit CETP raise blood pressure to an extent similar to that observed with torcetrapib.

Other CETP inhibitors such as dalcetrapib, anacetrapib and evacetrapib do not raise blood pressure.

Torcetrapib induces synthesis and secretion of both aldosterone and cortisol from human adrenal cells in tissue culture.

Torcetrapib reduces expression of endothelial nitric oxide synthase mRNA and protein, reduces nitric oxide release, increases expression of endothelin-1 and induces endothelial dysfunction animals independent of CETP inhibition

Other CETP inhibitors such as dalcetrapib and anacetrapib do not have these off-target effects

Effects on Diabetic Control in ILLUMINATE

There were 6101 patients with type 2 diabetes in the ILLUMINATE trial.

Treatment with torcetrapib resulted in a highly significant improvement in diabetic control.

Effects of Atorvastatin (A) vs Atorvastatin Plus Torcetrapib (T/A) in Diabetics in ILLUMINATE (N = 6661)

Glucose

(p<0.0001 for all)

Plasma glucose (mmol/L)

Effects of Atorvastatin (A) vs Atorvastatin Plus Torcetrapib (T/A) in Diabetics in ILLUMINATE (N = 6661)

HbA1C \( (p<0.0001 \text{ for all}) \)

Effects of Atorvastatin (A) vs Atorvastatin Plus Torcetrapib (T/A) in Diabetics in ILLUMINATE (N = 6661)

Insulin

Effects of Atorvastatin (A) vs Atorvastatin Plus Torcetrapib (T/A) in Diabetics in ILLUMINATE (N = 6661)

HOMA-IR

## Lipid efficacy of CETP inhibitors (% change from baseline)

<table>
<thead>
<tr>
<th>CETP inhibitor</th>
<th>Dose mg/d</th>
<th>HDL-C %</th>
<th>LDL-C %</th>
<th>TG %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torcetrapib</td>
<td>60</td>
<td>61</td>
<td>-24</td>
<td>-9</td>
</tr>
<tr>
<td>Dalcetrapib</td>
<td>600</td>
<td>31</td>
<td>-2</td>
<td>-3</td>
</tr>
</tbody>
</table>

Adapted from Cannon C, JAMA 306:2154; 2011
**dal-HEART Program:**
dalcetrapib HDL Evaluation, Atherosclerosis & Reverse Cholesterol Transport

Double-blind, randomized, placebo-controlled studies

**dal-OUTCOMES**
15,872 patients recently hospitalized for ACS
CV outcomes

**dal-VESSEL**
476 patients with CHD or CHD risk equivalent
endothelial function and blood pressure
Completed

**dal-PLAQUE**
130 patients with CHD inflammation, plaque size and burden, measured by PET/CT and MRI
Completed

**dal-PLAQUE 2**
906 patients with CAD on atherosclerotic disease progression, assessed by imaging
Fully recruited

**dal-ACUTE**
300 patients hospitalized for ACS <1 week after ACS treatment initiated within 1 week of an ACS
Fully recruited

**dal-OUTCOMES 2**
20,000 patients with stable CHD, CHD risk equivalents or at elevated risk for CVD
CV outcomes

FPI (Feb 2012)

---

5. Roche, data on file.
Dal-PLAQUE was the first multicentre study using novel non-invasive multimodality imaging to assess structural and inflammatory indices of atherosclerosis as primary endpoints.

Co-primary endpoints were MRI-assessed indices (total vessel area, wall area, wall thickness, and normalised wall index [average carotid]) after 24 months and $^{18}$F-fluorodeoxyglucose PET/CT assessment of arterial inflammation within an index vessel (right carotid, left carotid, or ascending thoracic aorta) after 6 months, with no-harm boundaries established before unblinding of the trial.

130 patients (men and women) aged 18–75 years, with CHD or CHD equivalent

Co-primary endpoints were MRI-assessed indices (total vessel area, wall area, wall thickness, and normalised wall index [average carotid]) after 24 months and $^{18}$F-fluorodeoxyglucose PET/CT assessment of arterial inflammation within an index vessel (right carotid, left carotid, or ascending thoracic aorta) after 6 months

Dal-PLAQUE- Results

189 patients were screened and 130 randomly assigned to placebo (66 patients) or dalcetrapib (64 patients).

There was no evidence of harm in dalcetrapib treated subjects, with a possible beneficial vascular effects as evidenced by a reduction in total vessel enlargement over 24 months.

Dalcetrapib did not increase blood pressure and the frequency of adverse events was similar between groups.

It was concluded that these results provided comfort with a decision to proceed with a longer and much larger clinical endpoint trial.

Dal-VESSEL

This trial investigated the effects of dalcetrapib on endothelial function, blood pressure, inflammatory markers and lipids in patients with, or at risk of, coronary heart disease (CHD) in a double-blind randomised placebo-controlled trial

476 patients with CHD or equivalent HDL-C < 50 mg/dL

The primary outcome measures were change from baseline of flow-mediated dilatation of the right brachial artery at 12 weeks and the 24-hour ambulatory blood pressure at week 4.

Dal-VESSEL- Results

476 patients were randomised. Change in FMD was not significantly different from placebo after 12 and 36 weeks of treatment with dalcetrapib.

After 4, 24 and 36 weeks of treatment with dalcetrapib, CETP activity decreased by 51, 53 and 56% while at weeks 4, 12 and 36 HDL-C increased by 25, 27 and 31%. Dalcetrapib had no effect on LDL-C levels.

Ambulatory blood pressure was unaffected by dalcetrapib up to 36 weeks.

Biomarkers of inflammation, oxidative stress and coagulation were unaffected by dalcetrapib up to 36 weeks, although Lp-PLA2 levels increased by 17%.

Objective of the dal-OUTCOMES trial

• To compare the effects of dalcetrapib with placebo, added to evidence-based background therapy, on cardiovascular risk in patients with recent acute coronary syndrome
dal-OUTCOMES Trial

15,600 patients
4-12 weeks
after an index
ACS event

Statin therapy
to optimal
LDL-C level

Dalcetrapib 600 mg

Placebo

2.5-year follow-up

Primary End Point
CHD death, non-fatal MI, atherothrombotic stroke, unstable angina requiring hospitalization or resuscitated cardiac arrest

(Likely to report at ACC in 2013)

Entry criteria

• Age $\geq$ 45 years
• Acute coronary syndrome
• Evidence-based management of LDL-C
• No restriction on entry level of HDL-C
• Key exclusions: Triglycerides >400 mg/dl; treatment with niacin, fibrates, or bile acid sequestrants.
Outcome measures

- **Primary outcome composite (time to first occurrence):**
  - Coronary heart disease death
  - Non-fatal MI
  - Ischemic stroke
  - Hospitalization for unstable angina (with objective evidence of acute myocardial ischemia)
  - Cardiac arrest with resuscitation

- **Secondary outcome measures:**
  - All cause mortality
  - Coronary revascularization
Flow of patients in the trial

- 19,005 entered single blind run-in
- 15,871 patients randomized
- Withdrawal of consent or loss to follow-up: dalcetrapib 3.9%, placebo 3.3%
- At the 2nd pre-specified interim analysis, including 1135 (71% of projected) primary endpoint events, the DSMB recommended termination of the trial for futility.
- At termination, median follow-up 31 mo.
### Baseline characteristics
(all balanced between treatment groups)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (years)</td>
<td>60</td>
</tr>
<tr>
<td>Female</td>
<td>19%</td>
</tr>
<tr>
<td>Caucasian</td>
<td>88%</td>
</tr>
<tr>
<td>Region</td>
<td></td>
</tr>
<tr>
<td>Europe or Israel</td>
<td>50%</td>
</tr>
<tr>
<td>North America</td>
<td>32%</td>
</tr>
<tr>
<td>Cardiovascular risk factors</td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>68%</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>63%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>24%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>21%</td>
</tr>
<tr>
<td>Cardiac biomarker-positive qualifying (index) event</td>
<td>87%</td>
</tr>
<tr>
<td>Time from index event to randomization (days)</td>
<td>61</td>
</tr>
</tbody>
</table>
## Concurrent treatments
(all balanced between treatment groups)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCI or CABG for index event (before randomization)</td>
<td>91%</td>
</tr>
<tr>
<td>Statin</td>
<td>97%</td>
</tr>
<tr>
<td>Aspirin</td>
<td>97%</td>
</tr>
<tr>
<td>Clopidogrel, ticlopidine or prasugrel</td>
<td>89%</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>88%</td>
</tr>
<tr>
<td>ACE inhibitor or ARB</td>
<td>79%</td>
</tr>
</tbody>
</table>
Baseline lipids (mean)
(all balanced between treatment groups)

<table>
<thead>
<tr>
<th></th>
<th>mg/dl</th>
<th>mmol/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL cholesterol</td>
<td>76</td>
<td>1.96</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>42</td>
<td>1.09</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>134</td>
<td>1.51</td>
</tr>
</tbody>
</table>
HDL-C and LDL-C by treatment group

Data are mean ± 95% CI
Primary outcome* by treatment group

Coronary heart disease death, non-fatal MI, ischemic stroke, hospitalization for unstable angina, resuscitated cardiac arrest

Hazard ratio 1.04
(95% CI 0.93-1.16)
P = 0.52 by log rank test

* Coronary heart disease death, non-fatal MI, ischemic stroke, hospitalization for unstable angina, resuscitated cardiac arrest
## Risk of primary and secondary outcomes

<table>
<thead>
<tr>
<th>Event</th>
<th>Dalcetrapib (% at 3 years)</th>
<th>Placebo (% at 3 years)</th>
<th>Hazard Ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary composite</strong></td>
<td>9.2</td>
<td>9.1</td>
<td><strong>1.04 (0.93-1.16)</strong></td>
<td><strong>0.52</strong></td>
</tr>
<tr>
<td><strong>CHD death</strong></td>
<td>1.6</td>
<td>1.8</td>
<td><strong>0.94 (0.73-1.21)</strong></td>
<td><strong>0.66</strong></td>
</tr>
<tr>
<td><strong>Non-fatal MI</strong></td>
<td>5.9</td>
<td>6.0</td>
<td><strong>1.02 (0.89-1.17)</strong></td>
<td><strong>0.80</strong></td>
</tr>
<tr>
<td><strong>Unstable angina</strong></td>
<td>1.3</td>
<td>1.3</td>
<td><strong>0.91 (0.68-1.22)</strong></td>
<td><strong>0.54</strong></td>
</tr>
<tr>
<td><strong>Resuscitated cardiac arrest</strong></td>
<td>0.2</td>
<td>0.1</td>
<td><strong>1.41 (0.63-3.18)</strong></td>
<td><strong>0.40</strong></td>
</tr>
<tr>
<td><strong>Ischemic Stroke</strong></td>
<td>1.4</td>
<td>1.0</td>
<td><strong>1.25 (0.92-1.70)</strong></td>
<td><strong>0.16</strong></td>
</tr>
<tr>
<td><strong>All cause mortality</strong></td>
<td>3.1</td>
<td>3.4</td>
<td><strong>0.99 (0.82-1.19)</strong></td>
<td><strong>0.90</strong></td>
</tr>
<tr>
<td><strong>Coronary revascularization</strong></td>
<td>9.5</td>
<td>9.6</td>
<td><strong>1.00 (0.87-1.11)</strong></td>
<td><strong>0.97</strong></td>
</tr>
</tbody>
</table>
Systolic blood pressure and hs-CRP were slightly higher with dalcetrapib than placebo

With dalcetrapib, compared with placebo:

• Mean **systolic blood pressure** was 0.6 mm Hg higher (P<0.001)
• No effect on plasma aldosterone, bicarbonate, or potassium
• No difference in number of anti-hypertensive medications

• At 3 months of assigned treatment, median **hs-CRP** was 0.2 mg/L higher (P<0.001, based on ANOVA after log transformation)
Conclusions

• In patients with recent ACS, the CETP inhibitor dalcetrapib raised HDL-C by ~30% with minimal effect on LDL-C and had no effect on the risk of major cardiovascular events.

• HDL-C concentration did not predict risk in this study population.

• Slightly higher systolic blood pressure and C-reactive protein with dalcetrapib might reflect an adverse effect of inhibiting CETP.
Why did Dalcetrapib fail to Reduce CV Events?

The two most obvious explanations are:

(i) The increase in HDL-C concentration induced by dalcetrapib is not accompanied by an enhancement of the protective functions of HDL or

(ii) that the inverse relationship between HDL-C concentration and cardiovascular risk observed in population studies is an epiphenomenon rather than being reflective of an ability of HDL to protect against cardiovascular disease.
Lipid efficacy of CETP inhibitors (% change from baseline)

<table>
<thead>
<tr>
<th>CETP inhibitor</th>
<th>Dose mg/d</th>
<th>HDL-C %</th>
<th>LDL-C %</th>
<th>TG %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torcetrapib</td>
<td>60</td>
<td>61</td>
<td>-24</td>
<td>-9</td>
</tr>
<tr>
<td>Dalcetrapib</td>
<td>600</td>
<td>31</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Anacetrapib</td>
<td>100</td>
<td>138</td>
<td>-40</td>
<td>-7</td>
</tr>
</tbody>
</table>

Adapted from Cannon C, JAMA 306:2154; 2011
1620 patients with CHD or CHD risk equivalents

Statin therapy to achieve LDL-C <100 mg/dL

Anacetrapib 100 mg

Placebo

76 week follow-up

Primary End Point
Lipid efficacy and the safety

Cannon et al. NEJM. 2010; 363:2406.
DEFINE Trial

LDL-C (mg/dL)

Placebo

Anacetrapib

Study Week

HDL-C (mg/dL)

Anacetrapib

Placebo

Study Week

Cannon et al. NEJM. 2010; 363:2406.
DEFINE Trial

ApoB (mg/ml)

ApoA-I (mg/ml)

Cannon et al. NEJM. 2010; 363:2406.
DEFINE Trial

TG (mg/dL)

Placebo

Anacetrapib

Non-HDL-C (mg/dL)

Placebo

Anacetrapib

Cannon et al. NEJM. 2010; 363:2406.
DEFINE Trial

Lp(a) (nmol/L)

CRP (mg/L)

Cannon et al. NEJM. 2010; 363:2406.
Anacetrapib had no Effect on BP

Cannon et al. NEJM. 2010; 363:2406.
Adjudicated CV Events and Death

<table>
<thead>
<tr>
<th>Event</th>
<th>Anacetrapi N=808 n (%)</th>
<th>Placebo N=804 n (%)</th>
<th>Hazard ratio (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-specified adjudicated CV Safety endpoint</td>
<td>16 (2.0)</td>
<td>21 (2.6)</td>
<td>0.76 (0.39, 1.45)</td>
<td>0.40</td>
</tr>
<tr>
<td>Cardiovascular Death</td>
<td>4 (0.5)</td>
<td>1 (0.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fatal MI</td>
<td>6 (0.7)</td>
<td>9 (1.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>1 (0.1)</td>
<td>6 (0.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-fatal Stroke</td>
<td>5 (0.6)</td>
<td>5 (0.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Death from any Cause</td>
<td>11 (1.4)</td>
<td>8 (1.0)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Revascularization</td>
<td>8 (1.0)</td>
<td>28 (3.5)</td>
<td>0.29 (0.13, 0.64)</td>
<td>0.001</td>
</tr>
<tr>
<td>Death or major CV event</td>
<td>27 (3.3)</td>
<td>43 (5.3)</td>
<td>0.62 (0.38, 1.01)</td>
<td>0.048</td>
</tr>
</tbody>
</table>

Cannon et al. NEJM. 2010; 363:2406.
Primary Bayesian analysis revealed that the event distribution in DEFINE indicates a 94% predictive probability of dismissing a torcetrapib type increase in CV Events.

Cannon et al. NEJM. 2010; 363:2406.
ILLUMINATE Trial 2007 (torcetrapib)¹
DEFINE Trial 2010 (anacetrapib)²

ILLUMINATE Trial (2007)  N = 15,067 (torcetrapib)
Primary endpoint: MCVE
Revascularization

DEFINE trial (2010)  N = 1,623 (anacetrapib)
Primary endpoint: MCVE
Revascularization

REVEAL Trial
Randomized Evaluation of the Effects of Anacetrapib through Lipid-modification

30,000 patients aged > 50 with occlusive arterial disease

Atovastatin to achieve LDL-C target

Anacetrapib 100 mg
Placebo

Sites in North America, Europe and Asia

Primary End Point
Coronary death, myocardial infarction or coronary revascularization

4 year follow-up

Planned completion in 2017

www.revealtrial.org
Lipid efficacy of CETP inhibitors (% change from baseline)

<table>
<thead>
<tr>
<th>CETP inhibitor</th>
<th>Dose mg/d</th>
<th>HDL-C %</th>
<th>LDL-C %</th>
<th>TG %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Torcetrapib</td>
<td>60</td>
<td>61</td>
<td>-24</td>
<td>-9</td>
</tr>
<tr>
<td>Dalcetrapib</td>
<td>600</td>
<td>31</td>
<td>-2</td>
<td>-3</td>
</tr>
<tr>
<td>Anacetrapib</td>
<td>100</td>
<td>138</td>
<td>-40</td>
<td>-7</td>
</tr>
<tr>
<td>Evacetrapib</td>
<td>500</td>
<td>129</td>
<td>-36</td>
<td>-17</td>
</tr>
</tbody>
</table>

Adapted from Cannon C, JAMA 306:2154; 2011
Study Design

- Subjects with elevated LDL-C or low HDL-C
- Up to 8 week dietary lead-in period and withdrawal of lipid-modifying therapies
- 12 week treatment period
  - Evacetrapib (30, 100 or 500 mg) or placebo
  - Evacetrapib 100 mg or placebo in combination with statin therapy (simvastatin 40 mg, atorvastatin 20 mg, rosvastatin 10 mg)
- Co-primary endpoints: Percent change in HDL-C and LDL-C

Percent Change in HDL-C and LDL-C

**HDL-C**
- Placebo: -3.0%
- 30 mg: 53.6%*
- 100 mg: 94.6%*
- 500 mg: 128.8%*

**LDL-C**
- Placebo: 3.9%
- 30 mg: -13.6%*
- 100 mg: -22.3%*
- 500 mg: -35.9%*

* P<0.001 compared with placebo

Percent Change in HDL-C: Evacetrapib Plus Statin

<table>
<thead>
<tr>
<th>Statin</th>
<th>Dose</th>
<th>Percent Change in HDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simvastatin</td>
<td>40 mg</td>
<td>7.3%</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>20 mg</td>
<td>1.4%</td>
</tr>
<tr>
<td>Rosavastatin</td>
<td>10 mg</td>
<td>5.5%</td>
</tr>
<tr>
<td>Statin plus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evacetrapib</td>
<td>100 mg</td>
<td>86.6%*</td>
</tr>
<tr>
<td>Placebo</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P<0.001 compared with placebo

Percent Change in LDL-C: Evacetrapib Plus Statin


* P<0.001 compared with placebo
Evacetrapib Conclusions

• Evacetrapib monotherapy produced a dose-dependent increase in HDL-C up to 128.8% and decrease in LDL-C up to 35.9%.

• Significant incremental HDL-C and LDL-C changes were observed when evacetrapib 100 mg was administered in combination with statins.

• Evacetrapib was well tolerated with no evidence of adverse blood pressure or mineralocorticoid effects.

• The impact of evacetrapib on cardiovascular events remains to be determined.

Future of CETP Inhibition as a Strategy to Reduce CV Risk

- There is still a compelling case for further testing the hypothesis that inhibiting CETP by will be anti-atherogenic in humans so long as the hypothesis is tested using potent CETP inhibitors that more than double the level of HDL-C and reduce LDL-C by more than 30%.

- This hypothesis is currently being tested in large CV clinical endpoint trials.
CETP Inhibition: Effect on RCT?
Novel HDL Therapeutics

- Elevate plasma HDL-C
- Enhance cholesterol efflux and reverse cholesterol transport
Anti-atherogenic HDL functions

HDL

Anti-oxidant
Anti-inflammatory
NO-promoting
Anti-thrombotic
Anti-atherogenic HDL functions

**HDL**

- Anti-oxidant
- Anti-inflammatory
- NO-promoting
- Anti-thrombotic

**Cholesterol efflux and reverse cholesterol transport**
Reverse Cholesterol Transport

Liver

Bile

FC

CE

A-I

FC

LCAT

A-I

Macrophage

ABCG1

ABCA1

BL
Quantitation of macrophage reverse cholesterol transport in vivo

Genetic and pharmacologic interventions

Feces

Bile

Plasma

Macrophage

$^{3}\text{H}-\text{cholesterol, AcLDL}$

$^{3}\text{H-BA}$

$^{3}\text{H-FC}$

$^{3}\text{H-Chol}$
Measuring HDL Cholesterol Efflux Capacity

After George Rothblat, et al
Promoting Cholesterol Efflux and Reverse Cholesterol Transport

- apoA-I infusion
Normal Apo A1 and Apo A1 Milano Dimer

A1 = apolipoprotein A1
A1m = apolipoprotein A1 Milano

LCAT = lecithin cholesterol acyl-transferase

Lipid Binding In Vivo Catabolism

Can reconstituted HDL remove cholesterol from plaque?
Recombinant apoA-I Milano Produced Regression of Coronary Atherosclerosis

ETC-216: apoA-I milano/phospholipids

Copyright © 2003 American Medical Association.
Reconstituted HDL: The ERASE Trial

Percent Change in Atheroma Volume from Baseline to 6 weeks

- CSL-111: -3.4% (p = 0.48)
- Placebo: -1.6%

Plasma Selective Delipidation: LS-001 Study

Waksman et al. JACC. 2010;55;2727.
Effects of apoA-I vs LDL Interventions on Coronary Atherosclerosis by IVUS

Intensive Statin Treatment
Up to 53% reduction LDL-C for 2 years

apoA-I Milano or r-HDL for 4-5 weeks

-5.00
-2.50
0
+2.50
+5.00

Median Change in % Atheroma Volume

apoA-I Milano
JAMA 2003

Intensive Statin Treatment
REVERSAL
pravastatin 40 mg
540 days

Progression

VS

Delipidated HDL
ERASE
JAMA 2007

ASTEROID
rosuvastatin 40 mg
720 days

Mean LDL-C

50 60 70 80 90 100 110 120

Courtesy of Dr. Jan Johansson

Promoting Cholesterol Efflux and Reverse Cholesterol Transport

- apoA-I infusion
- apoA-I upregulation
Efficacy of RVX-208
Time Course of Changes in HDL and Large Particle HDL

Copyright © 2011 American College of Cardiology Foundation.
Regulation of Macrophage Cholesterol Efflux

ABCA1=ATP-binding cassette transporter A1; A1=apolipoprotein A1; FC=free cholesterol; LXR=liver X receptor; RXR=retinoid X receptor; PPAR=peroxisome proliferator-activated receptor; TZD=thiazolidinedione

Oxysterols

LXR/RXR

PPAR\(\alpha\), PPAR\(\gamma\), PPAR\(\delta\)

ABCA1

FC

A1
Promoting Cholesterol Efflux and Reverse Cholesterol Transport

- apoA-I infusion
- apoA-I upregulation
- LXR agonism
Targeting LXR

ABCA1=ATP-binding cassette transporter A1; A1=apolipoprotein A1; FC=free cholesterol; LXR=liver X receptor; RXR=retinoid X receptor; PPAR=peroxisome proliferator-activated receptor; TZD=thiazolidinedione
Effect of LXR Agonist on Macrophage to Feces Reverse Cholesterol Transport

LXR= Liver X receptor; BA= bile acid; FC= free cholesterol; AcLDL= acetylated low-density lipoprotein
An LXR Agonist Significantly Increased Macrophage-to-Feces Reverse Cholesterol Transport in vivo

Promoting Cholesterol Efflux and Reverse Cholesterol Transport

- apoA-I infusion
- apoA-I upregulation
- LXR agonism
- miR-33 antagonism
Targeting miR-33

- A1
- FC
- ABCA1
- LXR/RXR
- miR-33
- Anti-miR-33
Promoting Cholesterol Efflux and Reverse Cholesterol Transport

- apoA-I infusion
- apoA-I upregulation
- LXR agonism
- miR-33 antagonism
- LCAT infusion/activation
The HDL flux hypothesis