THE HDL HYPOTHESIS: IS IT ALIVE AND WELL OR IS IT ON LIFE SUPPORT?

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Models of apoA-I Containing HDL Particles


Framingham Heart Study

Risk of coronary heart disease in men aged 50-70 by LDL and HDL cholesterol levels

East Lansing, Michigan
Angiographic Effects of Lipid Drug Classes Meta-Analysis, 12 Trials

\[ \Delta \% = 3.0 - 0.076 \% \text{HDL-C} + 0.06 \% \text{LDL-C} \]

\[ R^2 = 0.96; P < 0.004 \]

S+N = simvastatin + niacin

*HATS (HDL - Atherosclerosis Treatment Study) data not shown in original study.


The HDL Proteome

Potential Antiatherogenic Actions of HDL
Odds Ratios for Coronary Artery Disease According to Efflux Capacity and Selected Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>1.92 (1.34-2.75)</td>
<td>0.00</td>
</tr>
<tr>
<td>Hypertension</td>
<td>1.80 (1.37-2.47)</td>
<td>&lt;0.00</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.30 (0.95-1.72)</td>
<td>0.10</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>1.01 (0.88-1.16)</td>
<td>0.99</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>0.85 (0.78-1.01)</td>
<td>0.06</td>
</tr>
<tr>
<td>Efflux capacity</td>
<td>0.75 (0.63-0.90)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Figure 1. Odds Ratios for Coronary Artery Disease According to Efflux Capacity and Selected Risk Factors. The logistic-regression model was also adjusted for age and sex. Odds ratios for continuous variables are per 1-SD increase.

Efficacy of Niacin Therapy in Patients Already Well Treated with a Statin

Atherothrombosis Intervention in Metabolic Syndrome with low HDL/high triglycerides: Impact on Global Health (AIM HIGH) Results

- 3414 subjects with CVD; mean age 64; 34% with T2DM and 71% with MeS; 94% had prior statin use
- Randomized to simvastatin ± ezetimibe to reduce LDL-C < 80 mg/dL, and then to niacin ER 2 gm (n = 1718) or PBO (n = 1696)

NEJM 2011;365;2255-67
**AIM HIGH: Baseline Lipids (mg/dL)**

<table>
<thead>
<tr>
<th></th>
<th>On Statin (n=3,196)</th>
<th>Off Statin (n=218)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C (mean)</td>
<td>71</td>
<td>119</td>
</tr>
<tr>
<td>HDL-C (mean)</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Triglycerides (median)</td>
<td>161</td>
<td>215</td>
</tr>
<tr>
<td>Non-HDL (mean)</td>
<td>107</td>
<td>165</td>
</tr>
<tr>
<td>Apo-B (mean)</td>
<td>81</td>
<td>111</td>
</tr>
</tbody>
</table>

**AIM HIGH: HDL-C at Baseline & Follow-up**

![Graph showing HDL-C levels over time for Combination Therapy and Monotherapy, with P < 0.001 for a significant difference.]

**AIM HIGH: Primary Outcome**

![Graph showing cumulative % with primary outcome over time for Combination Therapy and Monotherapy, with HR 1.02, 95% CI 0.87, 1.21, Log-rank P value 0.79, and 16.4% for Combination Therapy and 16.2% for Monotherapy.]

N at risk
- Monotherapy: 1696, 1581, 1381, 910, 436
- Combination Therapy: 1718, 1606, 1366, 903, 428
Effect of High Risk Groups on Primary Outcome

<table>
<thead>
<tr>
<th># Pts. with Events (% of Category)</th>
<th>ERN Better</th>
<th>ERN Worse</th>
<th>Hazard Ratio (95% CI)</th>
<th>P-val.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG ≥ 196 and HDL &lt; 33 *</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>46 (17.0)</td>
<td>54 (22.4)</td>
<td>0.74 (0.50, 1.09)</td>
<td>0.073</td>
</tr>
<tr>
<td>No</td>
<td>254 (16.3)</td>
<td>220 (15.1)</td>
<td>1.09 (0.91, 1.31)</td>
<td></td>
</tr>
<tr>
<td>TG ≥ 200 and HDL &lt; 32</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>40 (16.7)</td>
<td>50 (25.0)</td>
<td>0.63 (0.40, 0.96)</td>
<td>0.017</td>
</tr>
<tr>
<td>No</td>
<td>242 (15.2)</td>
<td>224 (14.8)</td>
<td>1.11 (0.93, 1.33)</td>
<td></td>
</tr>
</tbody>
</table>

*Highest tertile of TC and lowest tertile of HDL-C  **Heterogeneity by treatment

HPS2-THRIVE: Eligibility

Men and women
Aged 50-80 years
Prior history of: myocardial infarction;
    ischaemic stroke or TIA;
    peripheral arterial disease; or
diabetes with other CHD
No contra-indication to study treatments
No significant liver, kidney or muscle disease

Baseline LIPIDS on statin-based therapy

<table>
<thead>
<tr>
<th>Mean (SD) baseline</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>mg/dL</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>128 (22)</td>
</tr>
<tr>
<td>Direct-LDL</td>
<td>63 (17)</td>
</tr>
<tr>
<td>HDL</td>
<td>44 (11)</td>
</tr>
<tr>
<td>Triglycerides*</td>
<td>125 (74)</td>
</tr>
</tbody>
</table>

*64% fasted for >8 hours
The numbers of patients and % of category confused me. These appear to be for ERN vs placebo, but which group is which should be clarified. Same color coding should be used as in prior slide.
Effect of ERN/LRPT on MAJOR VASCULAR EVENTS

- Risk ratio 0.96 (95% CI 0.90 – 1.03)
- Logrank P=0.29

THERAPEUTIC MODULATION OF CHOLESTEROL ESTER TRANSFER PROTEIN

Polymorphisms in CETP and Risk for CHD

- In a genomewide analysis performed on 18,245 women enrolled in the Women’s Genome Health Study, the single nucleotide polymorphism Taq1B (rs708272) was associated with a per allele rise in HDL-C of 3.1 mg/dL and a 24% (95% CI, 0.62-0.94) reduction in risk for myocardial infarction (MI).
- Among men enrolled in the Veterans Affairs HDL Cholesterol Intervention trial, those with who were homozygous for the Taq1 B2B2 genotype had higher baseline levels of HDL-C and 48% lower rate of cardiovascular events compared to men with the B1B1 genotype.
- The Copenhagen City Heart Study demonstrated increased risk for CAD despite higher serum HDL-C in women with the loss of function polymorphism Ile405→Val, and a 36% lower risk for CAD in women with low HDL-C in the context of the gain of function mutations A373P and R451Q.
- In the Prevention of Renal and Vascular End-Stage Disease study, the E280C>A loss of function polymorphism was associated with reduced CETP activity, increased serum HDL-C, and increased risk for cardiovascular events.
**Polymorphisms in CETP and Risk for CHD**

- More recently, three important studies also suggest an inverse relationship between serum CETP activity and risk for cardiovascular events.
- In a ten year follow-up analysis of CAD patients treated with statins from the Regression Growth Evaluation Statin Study (REGRESS) study, it was shown that the Taq1B2 polymorphism is associated with reduced CETP activity and increased HDL-C. Interestingly, for each copy of the B2 polymorphism, risk for all-cause and coronary mortality increased by 30% and 53%, respectively.
- Both the Framingham Offspring Study and the Ludwigshafen Risk and Cardiovascular Health Study of German patients demonstrated an inverse relationship between CETP activity and risk for CAD-related events.

Kaplan-Meier curves showing survival free from CVD over the follow-up period in individuals above vs below the median plasma CETP activity

Vasan, R. S. et al. Circulation 2009;120:2414-2420

**CETP Inhibitors and Modulators**

![CETP Inhibitors and Modulators](image_url)
**Lipid Effects of CETP Inhibitors/Modulators**

% Change from Baseline

<table>
<thead>
<tr>
<th>CETP Agent</th>
<th>Dose (Mg/day)</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>Anacetrapib</td>
<td>100</td>
<td>138</td>
<td>-40</td>
<td>-7</td>
</tr>
<tr>
<td>Evecetrapib</td>
<td>500</td>
<td>129</td>
<td>-36</td>
<td>-11</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 et al. JAMA. 2011;306:2099-2109.

**Torcetrapib**

“Beneficial” Effects on Lipoproteins

<table>
<thead>
<tr>
<th>Phases</th>
<th>60 mg</th>
<th>90 mg</th>
<th>120 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>+/-1%</td>
<td>+/-7%</td>
<td>+/-8%</td>
</tr>
<tr>
<td>Torcetrapib</td>
<td>+/-42%</td>
<td>+/-49%</td>
<td>+/-55%</td>
</tr>
</tbody>
</table>

**Torcetrapib:** “Beneficial” Effects on Lipoproteins, but Increased Cardiovascular and Non-Cardiovascular Morbidity and Mortality

Patients Without Event (%)

0 100 200 300 400 500 600 700 800 900 1000

Days After Randomization

Is the toxicity of torcetrapib related to the mechanism or the molecule?

Off-target Pharmacological Effects of Torcetrapib

- In patients receiving torcetrapib in the ILLUMINATE trial there was a significant:
  - Increase in blood pressure:
    - 5.4 mmHg in SBP in the torcetrapib arm
    - >15 mmHg in SBP at 12 months in 9.4% of the atorvastatin-only group and 19.5% of the torcetrapib group (P < 0.001)
  - Decrease in serum potassium
  - Increase in serum bicarbonate
  - Increase in serum sodium
  - Increase in serum aldosterone
- The adverse outcomes in the ILLUMINATE trial may have been the consequence of off-target actions of torcetrapib and not related to CETP inhibition.1,2


Analysis of the Off-target Characteristics of Investigational CETP Inhibitors/Modulators

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Torcetrapib</th>
<th>Anacetrapib</th>
<th>Dalcetrapib</th>
<th>Evacetrapib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical evidence of increased BP</td>
<td>Yes1</td>
<td>No2</td>
<td>No3</td>
<td>No3</td>
</tr>
<tr>
<td>Preclinical evidence of increased aldosterone production*</td>
<td>Yes1</td>
<td>No3</td>
<td>No3</td>
<td>No3</td>
</tr>
<tr>
<td>Preclinical evidence of aldosterone synthase (CYP11B2) mRNA induction*</td>
<td>Yes4</td>
<td>No3</td>
<td>No3</td>
<td>No3</td>
</tr>
<tr>
<td>Preclinical evidence of RAAS-associated gene induction*</td>
<td>Yes5</td>
<td>No3</td>
<td>No3</td>
<td>No3</td>
</tr>
<tr>
<td>L-type Ca$^{2+}$ channel activation*</td>
<td>Yes6</td>
<td>No3</td>
<td>No3</td>
<td>No3</td>
</tr>
</tbody>
</table>


Dalcetrapib Phase IIb Trial

HDL-C Increase at Week 12

- **P < 0.0001 vs placebo**

NOTE: Dalcetrapib 600 mg is the dose used in phase III

Dalcestral (JTT-705) Attenuates Atherosclerosis In Rabbits

CETP Inhibitors & Stool Sterol Excretion

Although dalcestral provided a relatively smaller increase in HDL-C than did torcetrapib and anacetrapib, it was the most effective in promoting fecal excretion of microsomal triglyceride transfer protein (MTP), mainly as bile acids. In addition, dalcestral was shown to increase total fecal bile acid mass, which may be due to a higher turnover of HDL-C.


dal-HEART Program

Dalcestral HDL Evaluation, Atherosclerosis, and Reverse Cholesterol Transport

The dal-HEART Program tests a novel hypothesis that raising HDL through CETP inhibition will attenuate cardiovascular risk.

dal-OUTCOMES

A double-blind, randomized, placebo-controlled study in 15,650 patients recently hospitalized for ACS
Goal 1: To evaluate the effect of dalcestral on CV outcomes

dal-VESSEL

A double-blind, randomized, placebo-controlled study in 450 patients with CAD or CHD
Goal: To evaluate the effect of dalcestral on endothelial function and blood flow, measured by FMD and ABI

dal-PLAQUE

A double-blind, randomized, placebo-controlled study in 130 patients with CHD
Goal: To evaluate the effect of dalcestral on atherosclerotic plaque size and burden, measured by PET/CT and MRI

dal-PLAQUE 2

A double-blind, randomized, placebo-controlled study in 900 patients with CAD
Goal: To evaluate the effect of dalcestral on atherosclerotic progression, assessed by IVUS and carotid B-mode ultrasound
Dalcetrapib Phase III CVD Event Trials—Discontinued May 7, 2012

<table>
<thead>
<tr>
<th>Start Date</th>
<th>Projected End Date</th>
<th>Patient Population</th>
<th>Primary Endpoint</th>
<th>Study Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>April 2008</td>
<td>May 2013</td>
<td>N=15,600, acute coronary syndrome (ACS, clinically stable)</td>
<td>MACE</td>
<td>dal-OUTCOMES-I: No benefit—all-cause HEART Trials D/C’d</td>
</tr>
<tr>
<td>Feb 2012</td>
<td>Oct 2016</td>
<td>N=20,000 chronic CHD (standard 2nd prevention)</td>
<td>MACE</td>
<td>dal-OUTCOMES-II</td>
</tr>
</tbody>
</table>

Dalcetrapib failure: due to lack of robust ↑HDL-C vs other?


### dal-OUTCOMES Results: Isolated ↑HDL-C

![Graph showing isolated ↑HDL-C](image)


### dal-OUTCOMES Results: No ↓CVD

![Graph showing no ↓CVD](image)

Anacetrapib Effects on LDL-C and HDL-C

Baseline 6 12 18 24 30 46 62 76
HDL-C (mg/dL) (SE)
0 20 40 60 80 100 120
Anacetrapib
Placebo
Anacetrapib n = 776 757 718 687 647 607 572 543
Placebo n = 766 761 741 744 736 711 691 666

LDL-C
Study Week
Baseline 6 12 18 24 30 46 62 76
LDL-C (mg/dL) (SE)
0 20 40 60 80 100
Anacetrapib
Placebo
Anacetrapib n = 804 771 716 687 646 604 568 540
Placebo n = 803 759 741 743 735 711 691 666


Does Anacetrapib Reduce CVD Events?
DEFINE Results
Cardiovascular Events During the Treatment Phase of the Study

Cardiovascular Events
Prespecified, adjudicated cardiovascular safety end point
Death from cardiovascular causes
Nonfatal myocardial infarction
Hospitalization for unstable angina
Nonfatal stroke
Death from any cause
Heart failure
Revascularization
PCI
CABG

Hard CVD
No net \( \Delta \)

Soft CVD
74%

N=30,000, prior CVD; recruiting in North America, Europe, and Asia
Background LDL-C lowering with atorvastatin
Randomized to anacetrapib 100 mg/d vs placebo
Scheduled follow-up: 4 years (started 6/2011, estimated completion 1/2017)
Primary outcome: Cor. death, MI, or cor. revasc

www.revealtrial.org
http://clinicaltrials.gov/show/NCT01252953?term=anacetrapib&rank=4
**OTHER FAILURES**

- RVX-208, an Apo-AI production stimulator, failed to show benefit in reducing rates of coronary plaque progression by IVUS in the ASSURE trial.
- D-4F, an apo AI mimic, is now out of development.
- CSL-111, a native apo AL infusion failed to show significant impact on atheroscleros disease progression. CSL-112 remains in development.
- Apo AL(Milano) is no longer in development.
- Autologous delipidated HDL, LCAT activators, endothelial lipase inhibitors, LXR/FXR agonists, niacin receptor agonist remain in development.

**CONCLUSIONS**

- A low serum HDL-C is among the most important risk factors for CAD.
- At the present time, HDL-C is not a target of therapy.
- The “HDL hypothesis” (i.e., that raising HDL-C, HDL particles, or modulating HDL functionality impacts risk for CV events) is a matter of intensive investigation.
- A variety of new pharmacologic interventions are being developed that:
  - Increase serum HDL particle concentration by infusing autologous delipidated HDL, human native apoA-I and and apoA-I mimetics.
  - The regulation of RCT is being studied by agonizing nuclear transcription factors (LXR-alpha and FXR) and modulating the activity of enzymes responsible for HDL metabolism in serum (LCAT, CETP, endothelial lipase).

- A variety of pharmacological approaches are being developed to therapeutically modulate serum levels of HDL-C.
- Niacin should not be used in patients with optimal LDL-C/non-HDL-C.
- One controversial approach to HDL raising is the use of molecules that inhibit the activity of cholesteryl ester transfer protein (CETP), an enzyme involved in neutral lipid transfer between lipoproteins.
- Based on a number of considerations, including the complex relationship between loss of function mutations in CETP and risk for CAD and the clinical experience with torcetrapib and dalcetrapib, it is difficult to predict if CETP inhibition with other agents will be associated with reductions in rates of atherosclerosis disease progression and risk for cardiovascular events.
- One persistent weakness in the clinical trial development program for CETP inhibitors is that it is still not clear what degree of CETP inhibition is optimal for the successful prevention of secondary events and in what types of genetic/metabolic backgrounds and lipid profiles.
CONCLUSIONS

• Gene therapy is being tested in a murine model to evaluate the safety and efficacy of an antisense molecule to miR-33. Antisense technology is already being used in humans to treat familial hypercholesterolemia with mipomersen, an antisense oligonucleotide directed against the mRNA for apoprotein B.