HDL Review:
Translating new findings on LCAT into novel therapies

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Disclosures

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Overview

- HDL Biology
- LCAT Biology
- Phase I Clinical trial of rLCAT
- Single Patient IND Treatment FLD
HDL Structure and Composition

Pre-beta Discoidal Shaped HDL

Initial Cholesterol Acceptor

Alpha Spherical Shaped HDL

Delivers Cholesterol to Liver
HDL Structure and Composition

Pre-beta Discoidal Shaped HDL

LCAT

Initial Cholesterol Acceptor

Alpha Spherical Shaped HDL

Delivers Cholesterol to Liver
HDL-Proteome

Vaisar T, et al JCI 2007
Pleiotropic Anti-Atherogenic Effects Of HDL ("Good Cholesterol")

- Antiinflammatory Activity
- Reverse Cholesterol Transport
- Antithrombotic Activity
- Antioxidative Activity
- Antipoptotic Activity
- Epithelial Repair
- Vasodilatory Activity

Pleiotropic Anti-Atherogenic Effects Of HDL ("Good Cholesterol")

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- Antioxidative Activity
- Epithelial Repair
- Vasodilatory Activity

Reverse Cholesterol Transport Pathway

ApoA-I

Liver

ABCG5/G8
LDL-R
ABCA1
SR-B1

Biliary Cholesterol + Bile Salts

CETP

CE

LDL

Stool

Pre-β HDL

α₁-₃ HDL

α₄ HDL

LCAT

Macrophage

ABCA1
ABCG1
SR-B1
Anti-Atherogenic Effects of HDL

- Inhibition of monocyte-adhesion
- Inhibition of LDL-oxidation
- Cholesterol-efflux

HDL affects:
- Endothelium
- Monocytes
- LDL
- Ox-LDL
- Macrophages
- Foam cell
Management of low HDL-C

- Therapeutic lifestyle changes
- Pharmacologic therapy
  - Statins
  - Fibrates
  - Niacin
  - CETP-inhibitors ?
  - Acute HDL Therapy ?
ApoA-I Milano Infusion Studies

Limone sul Garde, Italy

Free SH group
Improved anti-oxidant
ApoA-I Milano Infusion Studies

Limone sul Garde, Italy

Pre-clinical Animal Exp.

Free SH group
Improved anti-oxidant

Circ. 103(2001) 3047-50
Recurrent CVD in ACS Patients

Figure adapted from PLATO Trial
Cornel JH et al. Am Heart J 2012;164:334-342
Clinical trials of HDL Infusion Therapy

ApoA-I Milano (recombinant) Trial

- Change in atheroma volume

<table>
<thead>
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<th></th>
<th>Placebo (n = 11)</th>
<th>15 mg/kg (n = 21)</th>
<th>45 mg/kg (n = 15)</th>
<th>Combined (n = 36)</th>
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<td>mm³</td>
<td>-2.9 ± 23.3</td>
<td>-15.1 ± 50.6</td>
<td>-12.6 ± 15.3</td>
<td>-14.1 ± 39.5</td>
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<tr>
<td>p value</td>
<td>0.97</td>
<td>0.02</td>
<td>0.007</td>
<td>&lt;0.001</td>
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</table>

ETC-216

CSL-111 (Purified ApoA-I) Trial

Borders traced at every 1 mm in the 30-mm coronary segment of interest at baseline and follow-up

Overview

- HDL Biology
- LCAT Biology
- Phase I Clinical trial of rLCAT
- Single Patient IND Treatment FLD
Predicted Structure of LCAT Protein

Alpha/Beta Fold Structure

LCAT Features

Plasma protein produced by liver
Concentration 5 ug/mL
Mostly resides on HDL but also LDL
Over 75 mutations described
FLD mutations tend to decrease LCAT to a greater extent and affect esterification on HDL and LDL
FED mutations tend to affect esterification just on HDL

Vanloo B et al. J. Lipid Res. 2000;41:752-761
LCAT Reaction

Phosphatidylcholine + Lysophosphatidylcholine → LCAT → Lipoproteins → Cholesteryl Ester

nascent HDL → mature HDL
Potential Clinical Indications for rLCAT

- Acute coronary syndrome
  For the rapid stabilization of ACS patients

- Familial LCAT deficiency (FLD)
  For the prevention and or treatment renal disease.
LCAT Potentiates the Reverse Cholesterol Transport Pathway

- LDL
- LDL-R
- SR-BI
- CETP
- mature HDL (α-HDL)
- LCAT
- nascent HDL (Preβ-HDL)
- ABCA1
- ABCG1
- Cholesteryl Ester
- Free Cholesterol
Features of LCAT Deficiency

Clinical Features
- Rare autosomal recessive
- Cloudy cornea “fish eye”
- Normochromic Anemia
- End stage renal disease
- Complete Deficiency FLD
- Partial Deficiency FED

Lipoprotein Profile
- Low HDL-C (<10 mg/dL)
- Low apoA-I (20-30 mg/dL)
- <25% Cholesteryl esters
- Low LDL-C
- Presence of Lp-X particles
LpX and Renal Disease

**LpX Features**

± 60% PL  
± 30% Free Cholesterol  
≥ 5% Albumin  
No apoB or neutral lipids  
Some exchangeable apo’s  
30 – 70 nm  
1.038-1.058 g/mL

Bilayer or multi-lamellar complex of phospholipids

Aqueous core

Lp-X
**LpX Features**

± 60% PL  
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**Glomerulus in FLD**

**“Foamy” Mesangial cells**

**Accumulation Lipid in BM**

Bilayer or multi-lamellar complex of phospholipids

Aqueous core

Lp-X
LpX Cell Uptake Studies

Electron microscopy of LpX

Mesangial cell uptake of LpX

Lipoprotein Electrophoresis

LpX
LpX frozen x1
LpX frozen x2
Control plasma
FLD plasma
FLD plasma frozen x1
FLD plasma frozen x2

HDL
VLDL
LDL
Origin

Sudan Stain

Filipin Stain

Lp-X NBD
Lysotracker

DAPI
merge
LpX Activates the Inflammasome

Inflammasome Activation Pathway
LpX Activates the Inflammasome

Inflammasome Activation Pathway

- Lysosomal Rupture
- Frustrated Phagocytosis
- NLRP1/NALP1 Inflammasome
- NLRP3/NALP3 Inflammasome
- NLRC4/IPAF Inflammasome
- Caspase-1 Activation
- Unconventional Protein Secretion
  - (pro-IL-1α, FGF2, Bcl, Apo-1/Wilch-1, Annexin A2, Perioxidin-1, Thionein-1)
- Glycolysis
- Other Substrates
  - (Actin, FISUR, Parkin, Pyrin, Caspase-7)
- Cytokine Processing
  - (IL-1β, IL-18)
- Pyroptosis

- Toxins
  - (Aerolysin, Manotolysin, Nigerin, Valinomycin)

- UV-B

- K⁺ Efflux

- MDP

- Anthrax LT
LpX Activates the Inflammasome

Inflammasome Activation Pathway

- Unconventional Protein Secretion (pro-IL-1α, FGF2, BID, AIP-1/WD repeat, Annexin A2, Perioxidase-1, Thiolredoxin-1)
- Glycolysis
- Other Substrates (Actin, PITYRI, Parkin, Pyrin, Caspase-7)
- Cytokine Processing (IL-1β, IL-18)
- Pyroptosis
- Caspase-1 Activation
- NLRC4/IPAF Inflammasome
- NLRC3/NALP3 Inflammasome
- NLRC1/NALP1 Inflammasome

Graphs showing:
- % cells with destabilized lysosome
  - Untreated
  - LpX
  - Untreated
  - HDL
- Log graph from 10^3 to 10^6 with M2 peak

Graph showing LpX, LpX-chol, and Control with a count axis.
LpX Activates the Inflammasome

Inflammasome Activation Pathway

- LpX activates caspase-1
- MDP stimulation
- LpX interacts with inflammasomes
- IL-1β production

Graphs showing:
- % cells with destabilized lysosome
- Cell count with M2 macrophages
- IL-1β levels

K+ Efflux
Toxins (Aerolysin, Maitotxin, Nigericin, Valinomycin)
K+ Low
MMP
MDP
Anthrax LT
LPS
PGI2
ROS
NLRP1/NALP1 Inflammasome
NLRP3/NALP3 Inflammasome
NLRC4/IPAF Inflammasome
Cytoplasmic Fragmentation
Caspase-1 Activation
Cathelin B
ROS
Type III/IV Secretion
Cytokine Processing (IL-1β, IL-18)
Pyroptosis
Other Substrates (Actin, FTSU4, Parkin, Pyrin, Caspase-7)
Glycolysis
Unconventional Protein Secretion (pro-IL-1α, FGF2, BID, AIP-1/NAIP-1, Annexin A2, Perioxidase-1, Thioridoxin-1)
Lysosomal Rupture
Frustrated Phagocytosis
UV-B
(PSU, CPPD, Silica, Asbestos, Aluminum Salts, β-Amyloid)
LpX and Renal Disease

Proteinuria (Albumin/creat. ratio)  Fold Increase in Nephrotoxic Genes

LpX injection in LCAT KO mice causes proteinuria and induces genes associated with nephrotoxicity.
Increased Pre-beta HDL and Decreased LCAT in CHD Subjects

LCAT Activity low in CHD

From Copenhagen City Heart Study

CHD-Red
NO-CHD Green
High HDL>62 mg/dL
Low HDL<33 mg/dL

Increased Pre-beta HDL and Decreased LCAT in CHD Subjects

From Copenhagen City Heart Study

CHD-Red
NO-CHD Green
High HDL>62 mg/dL
Low HDL<33 mg/dL

Increased Pre-beta HDL and Decreased LCAT in CHD Subjects

LCAT Activity low in CHD

Pre-beta HDL high in CHD

From Copenhagen City Heart Study

CHD-Red
NO-CHD Green
High HDL>62 mg/dL
Low HDL<33 mg/dL

Carotid MRI of LCAT Heterozygotes

- Similar lumen areas
- Approx. 30-fold increase plaque volume in FLD compared to controls
- No significant difference in CIMT

*Dulvenoorden R J Am Coll Cardiol (2011) 6:2481*
Similar lumen areas

Approx. 30-fold increase plaque volume in FLD compared to controls

No significant difference in CIMT

*Dulvenoorden R J Am Coll Cardiol (2011) 6:2481*
Production of Human Recombinant LCAT

- AlphaCore Pharma
- License/CRADA 2008
- HEK transfected cells
- 4-step purification following ZnCl ppt.
- > 99.5% purity
- Yield 15 mg/L from conditioned media

Intravenous rLCAT Rapidly Corrects Lipoprotein Profile in LCAT KO Mice

Intravenous rLCAT Rapidly Corrects Lipoprotein Profile in LCAT KO Mice

Intravenous rLCAT Rapidly Corrects Lipoprotein Profile in LCAT KO Mice

In Vitro Treatment of Serum with rLCAT Rapidly Normalizes FLD Lipoprotein Profile

FLD serum incubated 1 hour at 37° C with or without rLCAT

rLCAT Decreases Tissue Cholesterol Levels in LCAT Ko x hapoA-I Tg Mice

4 daily IP injections of rLCAT injection in LCAT KO x apoA-I Tg mice (N=5)  
* P<0.05
Aortic Gene Expression Changes after rLCAT Treatment

Favorable gene expression changes consistent with increased mobilization of cholesterol from arterial wall.
Synergistic Effect of rLCAT and HDL in Plasma Cholesterol Esterification (CER)

HDL(CSL-111) dose response

rLCAT dose response
Synergistic Effect of rLCAT and HDL in Plasma Cholesterol Esterification (CER)

Both HDL and LCAT are rate limiting

(* P<0.05 above baseline)
Synergistic Effect of rLCAT and HDL in Plasma Cholesterol Esterification (CER)

Both HDL and LCAT are rate limiting and show a synergistic effect on cholesterol esterification.

(* P<0.05 above baseline)
Co-infusion of rHDL and rLCAT Increases Cholesterol Efflux and Esterification in Mice

**Total Cholesterol**

- CSL111 (60mg/kg; n=4)
- CSL111 (60mg/kg) + hLCAT (30mg/kg; n=4)

**Increased Total Cholesterol**

- CSL111
- CSL111+LCAT

(* P<0.05)
Co-infusion of rHDL and rLCAT Increases Cholesterol Efflux and Esterification in Mice

**Total Cholesterol**

- CSL111 (60mg/kg); n=4
- CSL111 (60mg/kg) + hLCAT (30mg/kg); n=4

**Cholesteryl Ester**

- CSL111 (60mg/kg); n=4
- CSL111 (60mg/kg) + hLCAT (30mg/kg); n=4

**Increased Total Cholesterol**

(* P<0.05)

**Increased Cholesteryl Ester**

(* P<0.05)
Overview

- HDL Biology
- LCAT Biology
- Phase I Clinical trial of rLCAT
- Single Patient IND Treatment FLD
rLCAT Phase 1 Study Design

**Inclusion:**
- History of stable documented CAD
- HDL-C < 50 mg/dL for men and < 55 mg/dL for women

**Endpoints:**
- Primary: Safety and tolerability
- Secondary: PK and PD of ACP-501

**Screening**
- Day -28 to -1
- Assess entry criteria

**Treatment**
- Day 0
  - ACP-501 infusion (observed in hospital 24 hrs)
- Day 1
  - 4 cohorts (0.9, 3.0, 9.0 and 13.5 mg/kg)
  - n=16 subjects
- Day 2
  - Out patient after 24 hrs

**Follow-Up**
- Day 28
  - Clinic Visits on Days 3, 4, 5 and 8

Sex: 14 males and 2 females
Dx: (Pos. hx CHD or radiology or dyslipidemia)
Race: 12 Caucasian and 4 other races
Mean age: 67.6 years
Mean HDL-C: 37.6 mg/dL
Summary of rLCAT Safety Data

- All subjects completed the study
- No Serious Adverse Events
- No infusion reactions
- No clinically significant changes in clinical lab tests, PE, vital signs, EKG attributable to drug
- Two mild rashes possibly attributable to drug
Dose Response rLCAT vs. HDL-C

rLCAT acutely increased HDL-C in dose dependent manner
rLCAT Increases HDL-C and CE

rLCAT treatment increases HDL-C and CE, which peaks at 12-24 h but remains partially elevated after 7 days.
Non-denaturing Gel Electrophoresis of HDL after rLCAT Treatment

rLCAT transiently decreases pre-beta HDL and increases larger size HDL species.
rLCAT increases apoA-I but no effect on TG

rLCAT treatment also increases apoA-I but is delayed compared to HDL-C and persists longer
rLCAT transiently lowers LDL-C and then increases it by day 3.

Peak in HDL-C levels following rLCAT precedes rise in LDL-C consistent with CETP mediated transfer of CE from HDL to LDL.
rLCAT transiently decreases ABCA1 dependent cholesterol efflux from plasma and then later increases cholesterol efflux.
Compared to rHDL, ACP-501 caused a higher and more sustained increase in HDL-C and more CE formation.
Overview

- HDL Biology
- LCAT Biology
- Pre-clinical animal models
- Phase I Clinical trial of rLCAT
- Single Patient IND Treatment FLD
52-yo male  FLD patient recommended for dialysis

Stage 4/5 renal disease
BUN 159 mg/dL
Creatinine 5.56 mg/dL
cystatin-C 4.16 mg/L
Urine protein 2307 mg/24hr

Very low HDL-C < 5 mg/dL
Anemia
Hgb 8.2 g/dL
Hct 24.7%

Severe Corneal opacities
Safety Tolerability

- rLCAT was **well-tolerated** with 1 non-attributable serious adverse event of recurrent atrial fibrillation and **no injection site reactions/toxicities**.
Effect rLCAT on FLD Lipids

rLCAT treatment normalized lipoproteins and % CE normalized for more about 1 week after single treatment at higher doses.
Effect rLCAT on HDL Subfractions
One rLCAT treatment every 2 weeks significantly raised HDL-C above baseline and maintained cholesteryl esters above threshold which should prevent LpX formation.
One rLCAT treatment every 2 weeks significantly raised HDL-C above baseline and maintained cholesteryl esters above threshold which should prevent LpX formation.
rLCAT treatment appeared to modestly improve renal function.
Effect of rLCAT on Anemia

rLCAT improves significantly improves anemia by decreasing cholesterol content on RBCs
Summary rLCAT Clinical Trials

- rLCAT was safe and well-tolerated:
  - No Serious adverse events
  - 2 possibly attributable AEs: mild skin rashes
  - No clinically significant attributable changes in clinical lab tests, physical exam, ECG parameters or vital signs

- Plasma LCAT mass and activity increased with dose of rLCAT and returned to baseline by 48 hours
Summary rLCAT Phase I Clinical Trial

Evidence that RCT pathway is stimulated by rLCAT

- Rapid increases in HDL-C and cholesterol esterification
- Increased \textit{in vitro} cholesterol efflux by plasma
- Transient decrease in LDL-C followed by an increase suggestive of CETP transfer of esterified cholesterol

\begin{itemize}
  \item rLCAT reverses biochemical abnormalities in FLD and support its use in future clinical trials to prevent or reverse renal disease in FLD
\end{itemize}
THE MECHANISM OF
THE PLASMA CHOLESTEROL ESTERIFICATION REACTION:
PLASMA FATTY ACID TRANSFERASE*

JOHN A. GLOMSET
Division of Endocrinology and Metabolism, Department of Medicine,
University of Washington, Seattle, Wash. (U.S.A.)
(Received May 14th, 1962)
Collaborators

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- Lita Freeman
- Xavier Rousset
- Amar Sethi
- Seth Thacker
- Lusana Aslan
- Alice Ossoli

AstraZeneca
(Alpha Core Pharma)
- Bruce Auerbach
- Brian Kraus
- Reyn Homan
- Becky Baker

Vascular Strategies
- Ernie Schaeffer
- Bella Azlos
- Steve Adelman
Questions?

Why not just clean out your pipes with rLCAT Therapy?

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