Hypertriglyceridemia: Application of Genetic Insights to Diagnosis and Management

Clinical Lipid Update 2016
National Lipid Association
San Diego

Joseph L. Witztum, MD
University of California, San Diego

Disclosures for Joseph Witztum

Consultant:
Ionis Pharmaceutical (formerly Isis Pharm)
Cymabay
Intercept
Prometheus
Hypertriglyceridemia Prevalence
NHANES Data 1999-2008

Fasting Triglyceride
Prevalence

| ≥ 150 | 31.0 % |
| ≥ 200 | 16.2%  |
| ≥ 500 | 1.1%   |

Elevated TG levels have both monogenic and polygenic causes

TG levels >10 mmol/L, especially in younger patients, are more likely to be due to monogenic causes together with secondary factors.

TG levels between 2 and 10 mmol/L represent the interplay of multiple genes (both heterozygous mutations of large effect and cumulative burden of small effect variants).

Both monogenic and polygenic HTG are exacerbated by non-genetic secondary factors.

Hegele et al. Diabetes Endodo 2014
Apolipoprotein C-III
Key Regulator of Serum Triglyceride Levels

- ApoC-III is a 79 amino acid glycoprotein synthesized principally in the liver
  - Associated with apoB-containing lipoproteins and HDL
- Plays a key role in determining serum triglyceride (TG) levels
  - Potent inhibitor of LPL-catalyzed lipolysis of triglyceride rich lipoproteins
    - Inhibits lipoprotein lipase activation by apoC-II
    - Inhibits hepatic lipase which also plays an important role in the conversion of dense VLDL to IDL
    - Inhibits receptor-mediated uptake of lipoprotein remnants by the liver
- Independent risk factor for cardiovascular disease

ApoC-III in a complex with an SDS micelle as derived by NMR

ApoC-III is a Genetically Validated Target for Cardiovascular Disease Risk Reduction

- Individuals with loss of function mutations in ApoC-III exhibit a favorable lipid profile, reduced CHD and increased longevity
  - Old World Amish
  - Ashkenazi Jews
  - Exome Sequencing Project
  - Copenhagen City cohorts
  - The Multi-Ethnic Bioimage Study

Novel Antisense Therapy to ApoC-III Lowers TG

Overview of Triglyceride Rich Lipoprotein (TRL) Metabolism

- ApoC-III is a glycoprotein synthesized principally in the liver that plays a key role in determining serum triglyceride levels
- Inhibits clearance of triglycerides from the blood
ApoC’s: (ApoC-I, apoC-II and apoC-III) are made in liver and secreted into plasma on VLDL

Fig. 8. DEAE-cellulose chromatography of Sephadex Fraction S2 (Fig. 5). Protein concentration is indicated by solid line and conductivity of the buffer by dotted line. The elution volume is given on the abscissa in milliliters.

Fig. 9. Polyacrylamide gel electrophoresis of DEAE-cellulose fractions in 8% gels on 5% acrylamide.
<table>
<thead>
<tr>
<th>Current name</th>
<th>Initial name</th>
<th>Carboxy-Terminal</th>
<th>MW</th>
<th>Sialic Acid Molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo CI</td>
<td>D1</td>
<td>apoLP-Val</td>
<td>7,000</td>
<td></td>
</tr>
<tr>
<td>Apo CII</td>
<td>D2</td>
<td>apoLP-Glu</td>
<td>9,700</td>
<td></td>
</tr>
<tr>
<td>Apo CIII-1</td>
<td>D3</td>
<td>apoLP-Ala</td>
<td>9,700</td>
<td>1/1</td>
</tr>
<tr>
<td>Apo CIII-2</td>
<td>D4</td>
<td>apoLP-Ala</td>
<td>-</td>
<td>2/1</td>
</tr>
</tbody>
</table>


ApoC-II and even apoC-III activate LPL activity

HYPERTRIGLYCERIDEAMIA ASSOCIATED WITH DEFICIENCY OF APOLIPOPROTEIN C-II

W. Carl Breckenridge, Ph.D., J. Allee Little, M.D., George Steinbr, M.D., Anne Chow, B.Sc., and Mary Poapst, M.Sc.

Abstract A 59-year-old man with severe hypertriglyceridemia and no post-heparin lipolytic activity was studied because of a marked fall in plasma triglyceride concentrations after a blood transfusion. An apolipoprotein activator (apolipoprotein C-II) for lipoprotein lipase could not be detected by polyacrylamide-gel electrophoresis of apoproteins, immunodiffusion of the plasma against anti-apolipoprotein C-II or activation assays for lipoprotein lipase. Furthermore, the patient’s triglyceride-rich lipoproteins would not serve as substrate for lipoprotein lipase. The patient had latent post-heparin lipolytic activity, which appeared after the addition of apolipoprotein C-II to the post-heparin plasma. After a transfusion of 1 unit of plasma from a normal subject the patient’s plasma triglycerides fell within one day, from 1000 to 200 mg per deciliter and remained below preinfusion concentrations for six days. We conclude that this patient’s hypertriglyceridemia resulted from a deficiency of apolipoprotein C-II. (N Engl J Med 298:1265-1273, 1978)

Discovery of the biological importance of apoC-II in activation of LPL

Figure 5. Effect of Transfusions of Normal Plasma on the Plasma Triglyceride Concentrations of a Patient with Apolipoprotein C-II Deficiency.

Breckenridge et al. NEJM 1078
Apolipoprotein CIII (formally apoLp-Ala)

Isolation by gel filtration followed by DEAE chromatography.

Activation then inhibition at higher concentrations.

Pre-activation with optimum apo CII then inhibition with apo CIII.

Courtesy of Virgil Brown

Brown & Baginsky. BBRC 1972; 46:375-382
High Correlation between plasma apoC-III and TG
(Schonfeld,...and Witztum *Metabolism*, 1979)

\[ r = 0.92 \]
**ApoC-III on different Lipoproteins**  
(Schonfeld, ...and Witztum *Metabolism*, 1979)

![Graph showing ApoC-III on different Lipoproteins](image)

*Fig. 3.* Radioimmunoassay displacement curve produced by ApoC-III, in an assay containing R148-4, a rabbit antihuman ApoC-III antiserum, and 125I-ApoC-III. The parallel curves produced by VLDL and HDL₃ (d = 1.085–1.125), and the poor displacement by ApoC-II are also shown.

---

**High Correlation between plasma apoC-II and TG**  
(Schonfeld, ...and Witztum *Metabolism*, 1979)

![Graph showing High Correlation between plasma apoC-II and TG](image)

$r = 0.82$
Plasma levels of apoC-II and apoC-III in HLP
(Schonfeld, ...and Witzum Metabolism, 1979)

VLDL apoC-III > apoC-II (3 or 4:1)
As plasma TG goes up, more and more apoC-III is found on VLDL/chylos

Overview of Triglyceride Rich Lipoprotein (TRL) Metabolism

ApoC-III Inhibits clearance of triglycerides from the blood—chiefly by inhibiting LPL activity
ApoC-III is a Genetically Validated Target
CVD Directly Linked to Levels of ApoC-III in Human Populations

Polymorphism in ApoC-III gene in Ashkenazi Jews
- Lower ApoC-III levels
- Reduced blood pressure
- Improved insulin sensitivity
- Greater longevity

ApoC-III deficiency in Old World Amish
- Lower serum triglycerides
- Reduced cardiovascular disease
- Enhanced health & longevity

ApoC-III gene variants in Eastern Indian males
- ~ 30% average increase in ApoC-III levels
- ~ 60% increase in fasting plasma triglyceride concentrations
- Reduced triglyceride clearance
- Increased incidence of CVD, NASH & insulin resistance

Atzmon, et al. Plos Biology 2006; 4e

Loss of Function Mutations in the APOC3 Gene are Cardioprotective

Loss-of-Function Mutations in APOC3, Triglycerides, and Coronary Disease

The TG and HDL Working Group of the Exome Sequencing Project, National Heart, Lung, and Blood Institute®

Loss-of-Function Mutations in APOC3 and Risk of Ischemic Vascular Disease

Association of APOC3 LOF Gene Mutations with Risk of CHD among 110,970 Participants in 15 Studies

<table>
<thead>
<tr>
<th>Study</th>
<th>Ancestry</th>
<th>Odds Ratio (95% CI)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHI</td>
<td>EA</td>
<td>0.39 (0.14–0.89)</td>
<td>0.02</td>
</tr>
<tr>
<td>WHI</td>
<td>AA</td>
<td>0.09 (0.00–4.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>FH5</td>
<td>EA</td>
<td>0.50 (0.00–13.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>MDC-CVA</td>
<td>EA</td>
<td>1.20 (0.38–4.70)</td>
<td>0.96</td>
</tr>
<tr>
<td>ARIC</td>
<td>EA</td>
<td>0.59 (0.07–5.00)</td>
<td>0.76</td>
</tr>
<tr>
<td>ARIC</td>
<td>AA</td>
<td>2.40 (0.89–5.00)</td>
<td>0.05</td>
</tr>
<tr>
<td>IPM</td>
<td>EA</td>
<td>0.74 (0.32–1.60)</td>
<td>0.05</td>
</tr>
<tr>
<td>IPM</td>
<td>MA</td>
<td>0.31 (0.06–1.20)</td>
<td>0.34</td>
</tr>
<tr>
<td>IPM</td>
<td>AA</td>
<td>0.62 (0.32–1.20)</td>
<td>0.65</td>
</tr>
<tr>
<td>A163P_v1</td>
<td>EA</td>
<td>0.49 (0.17–1.00)</td>
<td>0.04</td>
</tr>
<tr>
<td>OHs</td>
<td>EA</td>
<td>0.35 (0.07–1.20)</td>
<td>0.10</td>
</tr>
<tr>
<td>PROCARDIS</td>
<td>EA</td>
<td>0.56 (0.22–1.30)</td>
<td>0.17</td>
</tr>
<tr>
<td>HUNT</td>
<td>EA</td>
<td>0.84 (0.54–1.30)</td>
<td>1.00</td>
</tr>
<tr>
<td>CoUDDTS CARD</td>
<td>EA</td>
<td>0.00 (0.00–1.40)</td>
<td>0.16</td>
</tr>
<tr>
<td>EPIC CAD</td>
<td>EA</td>
<td>1.00 (0.11–8.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>FAS</td>
<td>EA</td>
<td>0.00 (0.00–0.36)</td>
<td>0.002</td>
</tr>
<tr>
<td>German CAD</td>
<td>EA</td>
<td>0.54 (0.33–0.86)</td>
<td>0.007</td>
</tr>
<tr>
<td>WFCC</td>
<td>EA</td>
<td>0.08 (0.07–2.00)</td>
<td>1.00</td>
</tr>
<tr>
<td>All</td>
<td></td>
<td>0.60 (0.47–0.75)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>


Association of APOC3 LOF Gene Mutations with Risk of CHD among 110,970 Participants in 15 Studies

- 40% Reduction in apoC-III
- 40% Reduction in TG
- 40% Reduction in CHD Risk

Unsettled Questions

• The question remained if some of these data could be confounded by the possibility that the LDL-C levels were lower and that patients taking statins had not been accounted for.

• A new study just published however, examines these two questions, and also whether the association of apoC-III mutations applies to non-European populations.

Association of APOC3 Loss-of-Function Mutations With Plasma Lipids and Subclinical Atherosclerosis

The Multi-Ethnic Bioimage Study

<table>
<thead>
<tr>
<th>TABLE 1: ASSOCIATION OF APOC3 LOSS-OF-FUNCTION MUTATIONS WITH PLASMA LIPIDS AND SUBCLINICAL ATHEROSCLEROSIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Blood lipids:</td>
</tr>
<tr>
<td>Total cholesterol, mg/dL</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
</tr>
</tbody>
</table>

Subclinical atherosclerosis:

|                                                                      |                      |                  |                |        |         |
| Coronal atheroma burden, percentage of wall                         | 46.0 (0.0 to 245.5)  | 29.6 (0.0 to 227.5)| -22.9         | -51.1 to -4.7  | 0.019   |
| Carotid plaque, mm²                                                  | 183.8 (0.0 to 265.9) | 112.8 (0.0 to 267.2)| -8.7          | -73.5 to 56.0  | 0.79    |
| Carotid intima-media thickness, mm²                                 | 0.16 ± 0.10          | 0.14 ± 0.13      | -1.71          | -6.3 to 3.0    | 0.47    |

Natarajan et al JACC 2015
ApoC-III Loss of Function Mutations Associate with Decreased TG and Decreased Coronary Calcium in European and Non-European Populations

Summary:
Genetic lowering of apoC-III associated with:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>-43%</td>
<td>&lt;10^{-23}</td>
</tr>
<tr>
<td>HDL chol</td>
<td>+11%</td>
<td>&lt;10^{-10}</td>
</tr>
<tr>
<td>LDL chol</td>
<td>+1.5%</td>
<td>NS</td>
</tr>
<tr>
<td>CAC score</td>
<td>-28%</td>
<td>0.019</td>
</tr>
</tbody>
</table>

These associations were valid in both European and Non-European populations.

Natarajan et al JACC 2015

Hypertriglyceridemia is an Important CVD Risk Factor

TG Levels are Strongly and Positively Associated with CVD Risk

TG Levels are an Important “Residual Risk” Factor for CVD
Association of TG and CVD and All-Cause Mortality
Nordestgaard and Varbo Lancet 2014

Lowering TG has a Greater Absolute Risk Reduction Potential than Lowering LDL-C
PROVE IT-TIMI 22 Trial

CHD Event Rate After 30 Days, %

HR: 0.85 P=0.180
HR: 0.84 P=0.192
Referent

N=4162

LDL-C ≥70
LDL-C <70

TG <150
TG ≥150

15.0%
11.7%
16.5%
16.5%
17.9%
15.0%

15.0%
11.7%
16.5%
16.5%
17.9%
15.0%

Death, MI, and recurrent ACS
ACS patients on atorvastatin 80 mg or pravastatin 40 mg
Adjusted for age, gender, low HDL-C, smoking, hypertension, obesity, diabetes, prior statin therapy, prior ACS, peripheral vascular disease, and treatment
Lipid values are in mg/dL.

Mechanisms by Which ApoC-III Promote Atherosclerosis

1 Metabolic Effects promoting Foam cell formation

Elevating VLDL and IDL Levels

VLDL and IDL are found in the arterial intima—directly lead to foam cell formation.

Triglyceride Rich Lipoproteins (VLDL and IDL) Accumulate in Arterial Intima in Plaques

Fig. 5. Scanning graph of all plaque specimens. LDL-sized particles accounted for 56% of those counted, 30% were intermediate in size, and 14% were in the VLDL-size range.

Mechanisms by Which ApoC-III Promote Atherosclerosis

1. Metabolic Effects promoting Foam cell formation
   - Elevating VLDL Levels
     - VLDL and IDL are found in the arterial intima—directly lead to foam cell formation
   - Generation of more atherogenic LDL sdLDL

2. Direct activation of Endothelial Cells

3. Direct activation of Monocytes
Antisense Targeting of ApoC-III Demonstrates a Causal Role of ApoC-III in Hypertriglyceridemia-1

All of the epidemiological, genetic and Mendelian randomization studies provide strong suggestive evidence, but do not prove, a causal relationship between plasma apoC-III and hypertriglyceridemia, and in turn, decreased CVD.

The obvious way to determine if lowering apoC-III will in turn lower TG is to specifically lower apoC-III levels in plasma.

However, there is a reciprocal relationship between apoC-III and TG. Thus, lowering plasma TG by a number of different modalities, such as weight loss, and medications (fibrates, PPAR agonists, fish oils) in turn lower apoC-III levels—as well as other lipoproteins.

Thus, the ideal way to test this relationship is to directly inhibit hepatic synthesis of apoC-III.

Volanesorsen (ISIS 304801)
Ionis’ Advanced 2nd Generation ASO Chemistry
2’-O-(2-methoxethyl)-modified chimeric ASO

Chimeric RNase H1 ASO Design

<table>
<thead>
<tr>
<th>MOE</th>
<th>DNA</th>
<th>MOE</th>
</tr>
</thead>
</table>

↑ affinity  
↑ stability  
↑ tolerability  

RNase H1 Terminating Mechanism

Clinical Experience with 2nd Generation ASOs

- >5500 subjects treated by IV and/or SC administration
- >120 clinical studies
- Multiple therapeutic indications
- >100 patients dosed for >1 year
- Some patients dosed for > 4 years
- Doses up to 1200 mg
- Attractive tolerability profile

Specific sequence not repeated throughout genome, reducing potential for off-target binding
Volanesorsen inhibits apoC-III synthesis in the liver and therefore is very specific and if any other change are seen, one can be certain that these are due to inhibition of apoC-III alone.

Antisense inhibition of Apoc-III mRNA in a variety of animal models led to dose-dependent reductions of plasma apoC-III and TG levels.

ApoC-III inhibition did not decrease hepatic VLDL-TG secretion or intestinal TG secretion, but did increase plasma TG clearance.

Inhibition of apoC-III was not associated with hepatic TG accumulation or toxicity.

A phase-I study in healthy subjects demonstrated dose-dependent decreases in plasma apoC-III and concomitant lowering of TG and no safety signals.
Story 1
Targeting apoC-III in patients with FCS reveals new biology as to mechanisms by which apoC-III inhibits clearance of Triglyceride Rich Lipoproteins (TRL)

Story 2
Targeting apoC-III in non-FCS hypertriglyceridemia reveals a central role for apoC-III in regulating plasma TRL levels in hypertriglyceridemia of varied etiology

Triglyceride Entry and Clearance from Plasma
(Simplified View)
Triglyceride Entry and Clearance from Plasma
Prior Postulated Role of ApoC-III

LPL-dependent Clearance

ApoC-III (from VLDL and Chylomicrons)

LPL-independent Clearance

Familial Chylomicronemia Syndrome (FCS)
(Rare Genetic Syndrome)
Null loss-of-function mutations in LPL, GPIHPB1, APOC2, APOA5, or LMF1 genes

FCS absence of LPL activity
Familial Chylomicronemia Syndrome
Triglycerides > 1,000 mg/dL

Eruptive Xanthomas

Triglyceride Entry and Clearance from Plasma
Familial Chylomicronemia Syndrome

Volanesorsen (ISIS 304801)

ApoC-III

LPL-dependent Clearance

Liver (VLDL)

Intestines - Diet (Chylomicrons)

Liver Adipose tissue

Muscle

Fatty Acids (Fuel)
Mechanism of Hypertriglyceridemia in Human Apolipoprotein (Apo) C III Transgenic Mice
Diminished Very Low Density Lipoprotein Fractional Catabolic Rate
Associated with Increased Apo C III and Reduced Apo E on the Particles

Kamina Amo-Debrah, Edward A. Fisher, Xiaofan Chen, Toa Chajek-Shaul, Tony Hayes, Rudolf Zechner,
Annemarie Walsh, Rajasekhar Ramakrishnan, Henry N. Ginsberg, and Jan L. Breslow
Laboratory of Biochemical Genetics and Metabolism, The Rockefeller University, New York 10021; *Department of Physiology and Biochemistry, The Medical College of Pennsylvania, Philadelphia, Pennsylvania 19129; †Institute of Medical Biochemistry, Karl Franzens University Graz, A-8010 Graz, Austria; and ‡Department of Medicine, College of Physicians and Surgeons, Columbia University, New York 10032

Figure 4. The removal of labeled VLDL in a typical control (×), low (○), and high (●) expression mouse. Mice were injected with 200,000 dpm of in vivo radiolabeled VLDL. 60 μl of blood was drawn at each time point and the radioactivity of the serum sample was measured.

Triglyceride Entry and Clearance from Plasma
Study: ASO To Lower ApoC-III in FCS—Therapeutic Gamble

Volanesorsen (Ionis 304801)

Liver (VLDL)
Intestines - Diet (Chylomicrons)

??

ApoC-III

LPL-dependent Clearance

Plasma
TG

Fatty Acids (Fuel)

Liver
Adipose tissue
Muscle

LPL-independent Clearance

Normal
Targeting APOC3 in the Familial Chylomicronemia Syndrome

Daniel Gaudet, M.D., Ph.D., Diane Brisson, Ph.D., Karine Tremblay, Ph.D., Veronica J. Alexander, Ph.D., Walter Singleton, M.D., Steven G. Hughes, M.B., B.S., Richard S. Geary, Ph.D., Brenda F. Baker, Ph.D., Mark J. Graham, M.S., Rosanne M. Crooke, Ph.D., and Joseph L. Witztum, M.D.

Volanesorsen (ISIS 304801) was administered to 3 FCS patients (TG 1,406 - 2,083 mg/dL) in an open-label, 13-week study.

Gaudet et al. NEJM 2014

Volanesorsen Treatment Reduced Fasting Plasma ApoC-III Levels in FCS Patients (Absolute Levels)

Gaudet et al. NEJM 2014
Volanesorsen Treatment Reduced Fasting TGs in FCS Patients (Absolute Levels)

Fasting Triglyceride Levels

Gaudet et al. NEJM 2014

Postprandial TG and Chylomicron-TG Before and After Volanesorsen Rx

Gaudet et al. NEJM 2014
ApoC-III Regulates Both the LPL-dependent and LPL-independent Pathways of TRL Clearance

By Removing apoC-III Inhibition of the LPL-independent Clearance Pathway in FCS, TG Levels are Greatly Reduced
ApoC-III Inhibits Hepatic Clearance of Triglyceride-Rich Lipoproteins
Philip Gordts and Jeff Esko, UCSD

- ApoC-III on TRLs induces hypertriglyceridemia by preventing hepatic clearance via the LDLR/LRP1 axis.
- Syndecan-1 is crucial for clearing a unique set of TRLs enriched in ApoE, ApoA-V and ApoC-III

To Test the Hypothesis that apoC-III is a Central Regulator of TG Levels, We Treated Subjects with non-FCS Hypertriglyceridemia (TG 190-1,822 mg/dL) of Varied Etiologies

Liver (VLDL) Intestines - Diet (Chylomicrons)

ApoC-III

LPL-dependent Clearance

Plasma TG

Fatty Acids (Fuel)

Liver Adipose tissue Muscle

TG 200 --1,800

LPL-independent Clearance

ApoC-III
Volanesorsen (ISIS 304801) Therapy in Two Cohorts

Cohort 1: Monotherapy in subjects with mean TG of 581 mg/dL, n = 57
Cohort 2: Add-on to fibrate in subjects with mean TG of 376 mg/dL, n = 28
Volanesorsen Monotherapy Significantly Reduced TGs
(Mean % Change)

Gaudet et al. NEJM 2015

Volanesorsen Monotherapy Treatment Significantly Increased HDL-C
(Mean % Change)

Gaudet et al. NEJM 2015
Volanesorsen Treatment Significantly Reduced TGs as Monotherapy or in Combination with Fibrates

**Patients with baseline TG >750mg/dL**

### Summary of Patients Treated with Volanesorsen

**300 mg/week**

<table>
<thead>
<tr>
<th>Baseline Mean mg/dL [range]</th>
<th>Single Agent in Diabetics with High TG</th>
<th>Single Agent in Very High TG</th>
<th>In Addition to Fibrates in Very High TG</th>
<th>Single Agent in FCS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean % Change from Baseline (SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ApoC-III</td>
<td>-88% (5.4)</td>
<td>-80% (9.3)</td>
<td>-71% (13)</td>
<td>-81% (9.8)</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>-69% (10)</td>
<td>-71% (14)</td>
<td>-64% (8.9)</td>
<td>-69% (16)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>+42% (32)</td>
<td>+46% (24)</td>
<td>+52% (24)</td>
<td>+78% (75)</td>
</tr>
<tr>
<td>Non-HDL-C</td>
<td>-22% (18)</td>
<td>-11% (38)</td>
<td>-19% (29)</td>
<td>-58% (14)</td>
</tr>
</tbody>
</table>
Volanesorsen Improved Glucose Control

HbA1c Analysis in Diabetic Patients

Euglycemic Clamp
A Measure of Tissue Insulin Sensitivity

Important Added Benefit

Reduced ApoC-III Improved Glucose Control
- Decreased HbA1c
  - 1.22 percentage-point decrease (Pbo-adjusted)
- Improved Insulin Sensitivity
- Decreased:
  - Glycated Albumin
  - Fasting Fructosamine

Volanesorsen Phase 2 Safety and Tolerability Summary
Well Tolerated in Multiple Clinical Studies

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Drug Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALTs &gt;3x ULN</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Renal Function</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Biochemical labs</td>
<td>No Change</td>
<td>No Change</td>
</tr>
<tr>
<td>Flu-like Symptoms</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Injection site reactions</td>
<td>None</td>
<td>Infrequent/mild</td>
</tr>
</tbody>
</table>
APPROACH Trial (FCS Pivotal Study CS6)

Asialoglycoprotein Receptor (ASGPR)–GalNAc₃ Modified ASOs Allow 10-15x Drug Delivery to Hepatocytes

ISIS-APO(a)-LRx has 13X Improved Potency vs. Parent Molecule in Human Transgenic Apo(a) Mouse Model

Plasma Apo(a) Week 6

Log Dose (mg/kg/wk)

Percent Baseline (+/- SD)

-1.0 -0.5 0.0 0.5 1.0 1.5

ED$_{50}$ = 0.8 mg/kg/wk
ED$_{50}$ = 11 mg/kg/wk

494372 (MOE)
681257 (THA MBB)

ISIS-APO(a)-LRx Will Allow Great Flexibility in Dosing (> 30 fold more potent in humans than parent)

- Dramatic increase in potency supports dosing flexibility of weekly, monthly, quarterly or less frequently
- Quarterly dosing: steady state reduction of 80%, maximum reduction of >90%

Lp(a) mean % change from baseline (± SEM)

Observed Predicted

Nadir: > -90%

TIME (Months)

Approx. -45%

0 1 2 3 4 5 6 7 8 9

ISIS-APO(a)-LRx injection (20 mg)
PRX0081 ISIS-APO(a)-LRx
Volanesorsen-mediated Reduction in apoC-III Compared to Other TG Lowering Regimens

<table>
<thead>
<tr>
<th>Agent</th>
<th>ApoC-III Lowering (%)</th>
<th>TG Lowering (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volanesorsen</td>
<td>71-88</td>
<td>65-70</td>
</tr>
<tr>
<td>(minimal estimates)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrates</td>
<td>36</td>
<td>20-50</td>
</tr>
<tr>
<td>Niacin</td>
<td>30-35</td>
<td>20-50</td>
</tr>
<tr>
<td>Fish Oils</td>
<td>19-30</td>
<td>20-45</td>
</tr>
<tr>
<td>PPAR Agonists</td>
<td>33</td>
<td>30-40</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>10</td>
<td>10-15</td>
</tr>
<tr>
<td>Statins</td>
<td>20</td>
<td>7-30</td>
</tr>
</tbody>
</table>

Summary-1

- There is strong epidemiological, genetic, and Mendelian randomization data to support a key role of apoC-III in mediating elevated TG levels and increased risk for CVD.

- Prior to recent studies, it was thought that apoC-III’s major mode of raising TG was via inhibition of LPL activity.

- Consequent to conducting a high-risk, but hypothesis driven experimental study of ASO-mediated inhibition of apoC-III in FCS patients, who lack LPL activity, novel biology was learned: namely, that apoC-III inhibits LPL-independent pathways of TRL clearance, as well as LPL-dependent pathways.
These data demonstrate that apoC-III plays a central causative role in regulating plasma TRL levels.

Inhibition of hepatic apoC-III is a highly effective strategy to lower plasma TG levels in FCS patients, indicating the potential to prevent acute pancreatitis and other complications of very high TG, for which there is currently an urgent unmet need.

Inhibition of hepatic apoC-III was also highly effective in reducing plasma TG as well as non-HDL cholesterol and also in raising HDL-C in most hypertriglyceridemic subjects, indicating the potential to decrease the risk for CVD and to improve IR and the metabolic syndrome.

Thus, targeting apoC-III would appear to be an important strategy to reduce elevated TG levels and to test the hypothesis that reducing TG will reduce the associated risk of CVD.

These data indicate that antisense based therapy targeting hepatic apoC-III is a highly effective strategy to lower plasma TG in most hypertriglyceridemic subjects.

Volanersorsen, a current antisense agent targeting apoC-III was highly effective in lowering plasma TG levels in FCS patients, indicating the potential to prevent acute pancreatitis and other complications of very high TG, for which there is currently an urgent unmet need. It is currently undergoing a phase 3 trial in FCS.

GalNAc₃ modified ASOs show potential for a more potent compound with less frequent dosing in patient populations outside of FCS with high triglycerides and risk of CVD and should be available in the near future for clinical trials of the effectiveness of TG lowering to prevent CVC.
Daniel Steinberg MD, PhD
The Cholesterol Wars: The Skeptics vs the Preponderance of Evidence

• Hormone Sensitive Lipase
• Refsums Disease
• LRC “Coronary Primary Prevention Trial”
• Oxidized LDL and Role of Oxidation in Atherosclerosis
• Lowering the Age at which we begin to treat Cholesterol

Acknowledgements for Clinical Studies

Ionis Team
Vickie Alexander
Brenda Baker
Alex Bell
Rosanne Crooke
Richard Geary
Mark Graham
Steven Hughes
Richard Lee
Walter Singleton
Sotirios (Sam) Tsimikas
Nick Viney
Stan Crooke

Academic Team
Diane Brisson
John Brunzell
Daniel Gaudet
John Kastelein
Karine Tremblay

Akcea Team
Andres Digenio
Paula Soteropoulos