Clinical Trials
What’s Current and Why Participate

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Director, Women’s Preventive Cardiology BRRH
Disclosures: Research/Consulting/Advisory Boards/Speaking

- Amgen
- Sanofi/Regeneron
- BI/Lily
- Merck
- AstraZeneca
- Aralez
- Pfizer
- Esperion
- Madrigal
- Gemphire
Phases of Clinical Research

• **Phase 1**

  Phase I trials assess a medication’s safety. These trials also evaluate what the drug does to our bodies (pharmacodynamics) as well as what our bodies do to the drug (pharmacokinetics). This first phase of testing in human beings is performed in a very small number of healthy volunteers who and are ordinarily remunerated for their participation. A phase 1 trial will also investigate side effects and benefits that occur at various dosages. Phase 1 trials typically take several months to complete.
Phases of Clinical Research

• **Phase 2**
  
  After phase 1, when a medication has been shown to be safe, it is then further tested for efficacy. Phase 2 trials can take months to years to complete, and can involve several hundred subjects. These trials are typically randomized.

  • Sometimes these trials are double blinded.
Phases of Clinical Research

• Phase 3

• In a phase 3 trial, a drug is tested in hundreds to thousands of subjects. Thus, both pharmaceutical companies and the FDA gain a better understanding of the medication’s effectiveness, and safety. These trials are usually both randomized and double blinded, and can last several years. After a phase 3 trial has been successfully completed, pharmaceutical companies can request FDA approval to market the drug.

• When such a trial successfully gains FDA approval, it is often known as a “pivotal” phase 3 clinical trial.
Phases of Clinical Research

• **Phase 4**
  
  Phase 4 trials are performed with FDA approved medications in order to learn even more about an approved medication.
Top 5 Reasons people participate in Clinical Research

• To advance medicine (33%)
• To help improve the lives of others (29%)
• To help improve personal conditions (15%)
• To earn extra money (5%).
• To receive free medical care (3%).
Components of Clinical Research

- Research Site
  - Calibrated equipment
  - Locked storage for records and IP
  - Subjects
  - Principal Investigator
  - Sub-Investigators
  - Clinical Research Coordinators (CRC)
  - Clinical Research Assistants (CRA)
  - Regulatory Staff
- Contract Research Organization (CRO)
- Study Sponsor
- Pre-Site Visit (PSV)
- Site Initiation Visit (SIV)
- Frequent Interim Monitoring Visits (IMV)
20+ Active Clinical Studies (by indication)

- Hyperlipidemia (7)
- Statin Intolerance (2)
- Homozygous Familial Hypercholesterolemia (1)
- Hypertriglyceridemia (2)
- Familial Chylomicronemia Syndrome (1)
- Hyperlipoproteinemia(a) (1)
- Type 2 Diabetes (2)
- Heart Failure HFpEF & HFrEF (2)
- NASH/NAFLD (2)
Why Evaluate Ways to Lower LDL, TG, Lp(a), and Inflammation? Because they *Cause* ASCVD.

The evolution of association to causation, the history of LDL and other biomarkers
LDL-C, from Hypothesis to fact

“You can only know where you're going if you know where you've been.”

- James Burke
LDL and Cholesterol

• From Biology to Epidemiology to Randomized Clinical Outcomes Trials and Mendelian Randomization Studies
  • Evidence from outcomes studies shows a consistent linear relationship between the extent of low-density lipoprotein cholesterol (LDL-C) lowering with statin Rx (and other methods) and the relative reduction in risk of ASCVD events.

• Higher LDL-C = Higher CV Risk
• Lower LDL-C = Lower CV Risk

Understanding Cholesterol Metabolism

Where we’ve been
History of Cholesterol Understanding

1908–1913
Cholesterol related to atherosclerosis

1948
Framingham Heart Study begins

1961
Cholesterol identified as cardiovascular risk factor

1985
Brown and Goldstein awarded Nobel prize for cholesterol metabolism (LDL receptor)

1987
First statin approved

LDL-C and LDL-P are the End Products of Endogenous Lipoprotein Metabolism

- LDL receptors remove LDL from the circulatory system
- LDL, to a minor degree, delivers cholesterol to peripheral tissues

**Endogenous Pathway**

- LDL receptors
- Liver

**Exogenous Pathway**

- Chylomicron Remnants
- Intestine

HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; LPL = lipoprotein lipase; VLDL = very low-density lipoprotein

Cholesterol Plays a Role in Many Important Physiologic Functions

- Bile acid synthesis\(^1\)
- Component of myelin sheath\(^2,3\)
- Component of cell membranes\(^1,2\)
- Hormone and vitamin D synthesis\(^1,2\)
- Skin resistance to absorption of water-soluble substances\(^1\)

Cellular Acquisition of Cholesterol Can Be From Multiple Sources

• Cholesterol for cellular physiologic functions can be from intra and/or extracellular pathways\(^1\)–\(^5\)
  • Systemic distribution of cholesterol is important, but cells are not dependent on circulating plasma LDL-C\(^3\)

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HDLR = high-density lipoprotein cholesterol receptor; LDL-C = low-density lipoprotein cholesterol; LDL-R = LDL receptor; LDLRP = LDLR protein; SR-B1 = scavenger receptor class B type 1.


Figure adapted from Dietschy 2004.
Animal Data Demonstrate Steroidogenic Tissues Acquire Cholesterol via HDL-C and De Novo Synthesis\textsuperscript{1,2}

- Adrenal, ovarian, and testicular tissues can acquire cholesterol via LDL, HDL, and de novo synthesis
  - Predominant pathway is HDL and de novo synthesis\textsuperscript{1,2}

*Data were calculated from measurements made in 49-day-old control mice with LDL receptor activity.

HDL = high-density lipoprotein; LDL = low-density lipoprotein.
Despite Reducing LDL-C, Statins Do Not Alter Gonadal or Adrenal Steroid Hormones in Humans

Plasma hormone levels before and 3 months after treatment with statin

- Reduction in LDL-C with statins without changing steroid hormones has been consistently shown

*ng/mL for cortisol, DHEA-S and testosterone and nmol/L for SHBG. Effect of statin on gonadal and adrenal hormones studied on 24 patients with type 2 diabetes, studied before and after a 3-month treatment with statin.

DHEA-S = dehydroepiandrosterone sulfate; SHBG = sex hormone binding globulin.

The Central Nervous System Synthesizes Cholesterol De Novo

- The central nervous system synthesizes cholesterol de novo\(^1,2\)
- The blood–brain barrier prevents the uptake of systemic lipoprotein cholesterol\(^1,2\)
- This segregation ensures that cholesterol metabolism within the brain is isolated from changes in the circulating lipid levels\(^2\)

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Figure adapted from Katsuno M et al. 2009.
Our Evolving Understanding of LDL and Cholesterol Biology

• Knowledge of cholesterol’s role and trafficking mechanisms has dramatically evolved over the last century
• Cholesterol plays a role in various vital physiologic functions
• Cellular cholesterol is minimally dependent on extracellular acquisition; and not at all in the brain
• LDL is an insignificant source of cholesterol for steroid hormone synthesis
• Conflating LDL and cholesterol is a common and dangerous error: LDL ≠ Cholesterol
• In modern humans, LDL carries cholesterol destined for excretion
RCTs prove that lower LDL is better:
Statin effectiveness
Proportional effects on major vascular events per mmol/L LDL-C reduction

<table>
<thead>
<tr>
<th></th>
<th>No. of events (% pa)</th>
<th>Relative risk (CI) per mmol/L LDL-C reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statin/more</td>
<td>Control/less</td>
</tr>
<tr>
<td>More vs. less statin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PROVE-IT</td>
<td>406 (11.3)</td>
<td>458 (13.1)</td>
</tr>
<tr>
<td>A to Z</td>
<td>257 (7.2)</td>
<td>282 (6.1)</td>
</tr>
<tr>
<td>TNT</td>
<td>889 (4.0)</td>
<td>1164 (5.4)</td>
</tr>
<tr>
<td>IDEAL</td>
<td>938 (5.2)</td>
<td>1160 (6.3)</td>
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<tr>
<td>SEARCH</td>
<td>1347 (3.6)</td>
<td>1406 (3.8)</td>
</tr>
<tr>
<td>Subtotal (5 trials)</td>
<td>3837 (4.5)</td>
<td>4416 (5.3)</td>
</tr>
<tr>
<td>Statin vs. control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>First cycle (14 trials)</td>
<td>5883 (3.1)</td>
<td>7467 (4.0)</td>
</tr>
<tr>
<td>ALLIANCE</td>
<td>254 (5.4)</td>
<td>293 (6.4)</td>
</tr>
<tr>
<td>4D</td>
<td>144 (9.0)</td>
<td>162 (10.1)</td>
</tr>
<tr>
<td>ASPEN</td>
<td>114 (7.7)</td>
<td>136 (5.3)</td>
</tr>
<tr>
<td>MEGA</td>
<td>102 (0.5)</td>
<td>140 (0.7)</td>
</tr>
<tr>
<td>JUPITER</td>
<td>103 (0.5)</td>
<td>194 (1.0)</td>
</tr>
<tr>
<td>GISSI-HF</td>
<td>172 (2.2)</td>
<td>174 (2.2)</td>
</tr>
<tr>
<td>AURORA</td>
<td>382 (6.1)</td>
<td>368 (6.3)</td>
</tr>
<tr>
<td>Subtotal (21 trials)</td>
<td>7136 (2.8)</td>
<td>8934 (3.6)</td>
</tr>
<tr>
<td>Total (26 trials)</td>
<td>10973 (3.2)</td>
<td>13350 (4.0)</td>
</tr>
</tbody>
</table>

Difference between more vs. less and statin vs. control: $t = 4.5$, p < 0.001 (paired t-test)
IMPROVE-IT: EZ, Another piece of the puzzle

Statin and EZ trials show that LDL is causally related to vascular disease

But can we further prove causation?
Declaring Causation: the need for accuracy

• “Cardiology has a long history of finding a marker of a bad outcome and treating that marker of that bad outcome as if it were the cause of the bad outcome.” In other words, conflating association with causation.
  • David Brown, MD
Association doesn’t equal causation

• Grey hair is associated with ASCVD.
• Therefore, grey hair causes ASCVD.
• No: Older individuals have more grey hair and also a higher incidence of ASCVD events. This is an association, but not causal.
Reverse Causation, a common error in interpretation

• Low cholesterol is associated with cancer.
• Low cholesterol causes cancer.
• No: Cancer causes low cholesterol.
• This is reverse causation.
Mendelian Randomization (MR) Studies: Their Rationale

• Genotypes are unmodified by disease, limiting reverse causation.
• Genotypes are assigned randomly at meiosis, limiting confounding. (Mendel’s Law of Independent Assortment)
MR Requirements

1. The genetic variant affects the biomarker only. It does not impact other phenotypes.
2. The genetic variant associates with the disease in question in the same direction as the biomarker.
3. Exogenous (environmental) factors must be randomly distributed.
3 Components of a Mendelian Randomization Study

- “Instrument”: Gene variant that exclusively affects biomarker of interest; doesn’t affect other phenotypes (no confounding)
- “Theoretically predicted risk estimate”: based on amount of biomarker change, can accurately predict effect of variant on disease
- “Observed Risk Estimate”: Test variant for relationship with disease: Does the predicted estimate match the observed effect?
Figure 1  Conceptual background for Mendelian randomization studies: (A) Biomarkers 1–4 are associated with coronary artery disease but causality is unclear. Genetic variants and environmental factors affect the levels of these biomarkers. (B) Here a genetic variant not only associates statistically significant with the biomarker (+), but also with the complex disease. As a DNA variant has no immediate effect on disease manifestation, it can be expected that its effect on the biomarker acts as an indispensable intermediate step. Thus, the biomarker is causally involved in the disease process. (C) Here the genetic variant shows a sizable effect on the biomarker (+) but no association with coronary artery disease. Thus, it can be assumed that an equivalent variability of the biomarker has likewise no effect on disease risk; the biomarker is not causally involved in disease manifestation. (D) In this case exogenous factors influence the biomarker as well as coronary artery disease risk. Even if the genetic variant associates with the biomarker, its causal involvement in coronary artery disease cannot be assumed, since the single nucleotide polymorphism does not associate with coronary artery disease risk.
Figure 4 Brief overview about candidates tested in Mendelian randomization settings. While many biomarkers suggested a causal role in coronary artery disease in Mendelian randomization studies, others disappointed by negative results. The effect of diabetes mellitus single nucleotide polymorphisms was by far smaller than expected and barely significant. Numbers refer to the references in which the Mendelian randomization data have been reported. 100–105
SNP that lowers LDL, A MR Study
Association of rs2228671 with risk of CAD in six case-control studies

“LDL Hypothesis” is no longer “Hypothetical”; it is Reality

- LDL biology and pathophysiology
- Epidemiology
- Statin RCTs
- IMPROVE-IT
- FOURIER (To be discussed)
- Mendelian Randomization Studies
Enter PCSK9

PCSK9: Major modulator in LDL-C Metabolism
PCSK9 is a regulator of LDL metabolism

- Proprotein convertase subtilisin/kexin type 9 \(^1\)
- Secreted by liver into plasma \(^1\)
- Binds LDL receptor on surface of hepatocyte \(^1,2\)
- Targets LDL receptor for degradation \(^1,2\)

LDL particles are cleared from the plasma by binding to LDLR receptors and being internalized by the hepatocyte\textsuperscript{1-3}

PCSK9 binds to the LDL receptor, targeting it for degradation\textsuperscript{1-3}

Compared the incidence of CHD (MI, fatal CHD, or coronary revascularization) over a 15-year interval in the ARIC study according to the presence or absence of sequence variants in the PCSK9 gene that are associated with reduced plasma levels of LDL cholesterol.
Plasma LDL cholesterol levels and incidence of CHD in African Americans


n = 3,363 African American subjects

88% reduction in CHD risk
Plasma LDL cholesterol levels and incidence of CHD in Caucasians


n = 9,524 Caucasian subjects

47% reduction in CHD risk
Loss-of-Function Mutations in PCSK9 Are Associated With Decreased LDL-C

<table>
<thead>
<tr>
<th>PCSK9 Variant</th>
<th>Population</th>
<th>LDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>R46L</td>
<td>ARIC(^1), DHS(^2)</td>
<td>↓ 15(^1)%</td>
</tr>
<tr>
<td>Y142X or C679X</td>
<td>ARIC(^1), DHS(^2)</td>
<td>↓ 28(^1)–40(^1)%</td>
</tr>
<tr>
<td>R46L</td>
<td>CGPS(^3)</td>
<td>↓ 11(^3)%</td>
</tr>
</tbody>
</table>

- Heterozygous LOF mutations found in 1% to 3% of representative populations\(^1,3\)
- Associated with
  - Lower serum LDL-C\(^1\)
- PCSK9 null individual identified (compound heterozygote for two inactivating mutations)
  - No detectable circulating PCSK9 with strikingly low LDL-C (14 mg/dL)\(^4\)

LOF = loss of function
ARIC = Atherosclerosis Risk in Communities (N ~ 4,000); DHS = Dallas Heart Study (N = 3,553);
CGPS = Copenhagen General Population Study (N = 25,013)

# Gain-of-Function Mutations in PCSK9 Cause Familial Hypercholesterolemia*†

<table>
<thead>
<tr>
<th>PCSK9 Variant</th>
<th>Population</th>
<th>Clinical/Biochemical Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>D374Y¹</td>
<td>British, Norwegian families, 1 Utah family</td>
<td>Tendon xanthomas, severe hypercholesterolemia</td>
</tr>
<tr>
<td>S127R¹</td>
<td>French, South African, Norwegian families</td>
<td>Tendon xanthomas</td>
</tr>
<tr>
<td>R218S²</td>
<td>French families</td>
<td>Tendon xanthomas, arcus corneae</td>
</tr>
</tbody>
</table>

- Associated with:
  - High serum LDL-C¹
  - In vitro testing in many identified mutations shows decreased levels of LDLRs³

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*Autosomal Dominant Hypercholesterolemia


*For a full list of ADH mutations, please refer to Abifadel reference.*
Genetic Variants Establish PCSK9 as a Modulator of LDL-C

- Increasing PCSK9 (GOF)
  - Fewer LDL receptors$^{1,2}$
  - Higher LDL-C$^{1,2}$

- Decreasing PCSK9 (LOF)
  - More LDL receptors$^{1,2}$
  - Lower LDL-C$^{1,2}$

- Increased plasma levels of TC and LDL-C$^1$  
- FH-associated physical abnormalities$^1$

FH = familial hypercholesterolemia; GOF = gain of function; LDL-C = low-density lipoprotein cholesterol; LOF = loss of function; TC = total cholesterol.


**ExcelMedical CLINICAL TRIALS**
FOURIER Primary Endpoint

Hazard ratio 0.85
(95% CI, 0.79-0.92)
P<0.0001

Evolocumab
Placebo

CV Death, MI, Stroke, Hosp for UA, or Cor Revasc
Key Secondary Endpoint

Hazard ratio 0.80
(95% CI, 0.73-0.88)
P<0.00001
Landmark Analysis

- **16% RRR**
  - HR 0.84 (95% CI 0.74-0.96)
  - P = 0.008

- **25% RRR**
  - HR 0.75 (95% CI 0.66-0.85)
  - P < 0.00001
Fatal or Nonfatal MI or Stroke

- **19% RRR**
  - HR 0.81 (95%CI 0.70-0.93)
  - P=0.003

- **33% RRR**
  - HR 0.67 (95%CI 0.59-0.77)
  - P<0.00001

Months from Randomization

Evolocumab vs. Placebo
Comparison to Cholesterol Treatment Trialists Collaboration (CTTC)

Hazard Ratio (95% CI) per 1 mmol/L reduction in LDL-C

Major Coronary Events
- CTTC Meta-analysis Year 2
  - 0.78 (0.70-0.86)
  - 0.80 (0.71-0.90)

Stroke
- 0.77 (0.66-0.91)
- 0.77 (0.63-0.94)

Coronary revascularization
- Urgent
  - 0.73 (0.62-0.86)
- Elective
  - 0.84 (0.73-0.98)

Major Vascular Events
- 0.77 (0.73-0.82)
- 0.83 (0.76-0.90)

Lipid-lowering therapy better
0.5
1.0
Lipid-lowering therapy worse
2.0

CTTC data from *Lancet* 2010;376:1670-81
Comparison to Cholesterol Treatment Trialists Collaboration (CTTC)

Hazard Ratio (95% CI) per 1 mmol/L reduction in LDL-C

- **Major Coronary Events**
  - CTTC Meta-analysis Year 2: 0.78 (0.70-0.86)
  - FOURIER Year 2: 0.80 (0.71-0.90)

- **Stroke**
  - CTTC Meta-analysis Year 2: 0.77 (0.66-0.91)
  - FOURIER Year 2: 0.77 (0.63-0.94)

- **Coronary revascularization**
  - CTTC Meta-analysis Year 2: 0.75 (0.67-0.84)
  - FOURIER Year 2: 0.73 (0.62-0.86)
  - Urgent: 0.84 (0.73-0.98)

- **Major Vascular Events**
  - CTTC Meta-analysis Year 2: 0.77 (0.73-0.82)
  - FOURIER Year 2: 0.83 (0.76-0.90)

CTTC data from Lancet 2010;376:1670-81
<table>
<thead>
<tr>
<th>Safety</th>
<th>Evolocumab (N=13,769)</th>
<th>Placebo (N=13,756)</th>
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<tbody>
<tr>
<td><strong>Adverse events (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>77.4</td>
<td>77.4</td>
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<tr>
<td>Serious</td>
<td>24.8</td>
<td>24.7</td>
</tr>
<tr>
<td>Allergic reaction</td>
<td>3.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Injection-site reaction</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>Treatment-related and led to d/c of study drug</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td>Muscle-related</td>
<td>5.0</td>
<td>4.8</td>
</tr>
<tr>
<td>Diabetes (new-onset)</td>
<td>8.1</td>
<td>7.7</td>
</tr>
<tr>
<td>Neurocognitive</td>
<td>1.6</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Laboratory results (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Binding Ab</td>
<td>0.3</td>
<td>n/a</td>
</tr>
<tr>
<td>Neutralizing Ab</td>
<td><em>none</em></td>
<td>n/a</td>
</tr>
</tbody>
</table>

New-onset diabetes assessed in patients without diabetes at baseline; adjudicated by CEC
PCSK9 Summary

• PCSK9 regulates hepatic surface expression of LDLR and, in turn, systemic LDL-C levels

• Mutations in PCSK9 (both GOF and LOF) influence LDL-C levels as well as ASCVD risk

• PCSK9 is an excellent therapeutic target for LDL reduction

• Two PCSK9i were FDA approved the summer of 2015
  • But our prescriptions for this novel therapy were being consistently denied...
    And, now we know that evolocumab significantly reduced MI and CVA in FOURIER. But, denials persist!
Amgen 20140316
Study Design

Eligibility (N = 40)
- LDL-C apheresis x 3 mos (unchanged in last 4 wks)
- Tx goal LDL-C <100 mg/dL
- High/mod-intensity LLT
- Pre-apheresis LDL-C ≥100 mg/dL and ≤190 mg/dL
- Fasting TG ≤400 mg/dL

Primary endpoint:
Apheresis avoidance at end of randomized therapy (wks5-6)

Secondary endpoints:
Percent changes from baseline in LDL-C, non-LDL-C and CHOL/HDL-C

Clinicaltrials.gov (NCT02585895)
Lipoprotein Apheresis
Effective and “Comfortable”
End of Treatment: White Filter Becomes Yellow
LDL-Apheresis Demonstrated 72% Risk Reduction

Cholesterol Rebound After Receiving LDL Apheresis

LDL-C reductions with apheresis:
• Acute: Up to 76%¹
• Time averaged: 20-40%²

LDL-C (mg/dL) vs Treatments

Clinical Studies
1002-038-048 Bempedoic Acid

- LDL lowering with and without CVD
- LDL lowering in statin intolerance
- LDL lowering in different LLT combinations
Bempedoic acid: Lowering LDL-C

• Developing a differentiated oral therapy for treatment of patients with elevated LDL-cholesterol (LDL-C)
  – Works “like a statin”; but with reduced potential for muscle-related side effects
  – Targeting statin intolerance in patients with elevated LDL-C, a high unmet medical need

• **ETC-1002 (bempedoic acid) lowers elevated LDL-C**
  • Up to 30% as monotherapy and 50% in combination with ezetimibe
  • Incremental 24% LDL-C lowering when added to stable statin therapy

• **Lowers high-sensitivity C-reactive protein (hsCRP) up to 44%**

• **Appears safe and well tolerated**

• **Continuing progress on Phase 3 global development plans**
  – Robust Phase 1 & 2 program completed in >1,000 patients
  – Phase 3 global development plans to be formalized and communicated Q2 2016
Mechanism of action

**BEMPEDOIC ACID REDUCES LDL-C VIA INHIBITION OF ATP-CITRATE LYASE (ACL)**

Bempedoic acid is converted to ETC-1002-CoA in the liver which directly inhibits ACL, reduces cholesterol synthesis, and up-regulates LDL receptor activity.
Clinical Studies
Gemcabene

• LDL Lowering
• TG Lowering
Production Mechanism:
Gemcabene reduces production of cholesterol and triglycerides pathways inside the liver.

Clearance Mechanism:
Gemcabene clears VLDL efficiently due to a reduction in ApoC-III followed by rapid uptake by the remnant receptor.

- Plasma half life of 32 to 41 hours
- Liver is target organ
- Gemcabene is the active compound
- Renal elimination

*Potential molecular targets in the liver (ApoC-III, ACC)
Clinical Studies
UPENN FHGT002

AAV8-mediated low density lipoprotein receptor (LDLR) gene replacement in subjects with homozygous familial hypercholesterolemia (HoFH)

• Molecularly defined LDLR mutations in both alleles and clinical presentation consistent with HoFH
• No cardiovascular event or cardiovascular intervention within 12 weeks of enrollment
• A baseline serum AAV8 neutralizing antibody titer ≤ 1:10
Is AAV8-LDLR administration safe? Does it reduce LDL-C levels in HoFH?
Clinical Studies
Hyperlipoproteinemia(a) CS6

A randomized, double-blind, placebo-controlled, dose-ranging phase 2 study of ISIS 681257 administered subcutaneously to patients with hyperlipoproteinemia(a) and established cardiovascular disease (CVD)

- Lp(a) ≥ 60 mg/dL + CVD (documented CAD, stroke or PAD)
- Exclusions: Warfarin, direct thrombin inhibitors, factor Xa inhibitors (DOAC and NOACs)
Structure, Metabolism and Pathophysiology of Lp(a)

- Lp(a) MR studies prove ASCVD Causation

- Lp(a) is composed of apo(a) covalently bound to apoB-100 of LDL

- Plasma Lp(a) levels are >90% genetically determined.

- Apo(a) is produced in hepatocytes and is the primary determinant of plasma Lp(a) levels. Clearance of Lp(a) plays a secondary role.

- Currently, there is no effective therapy to potently and specifically lower Lp(a) levels

- Lp(a) is pro-atherogenic via three major components
  - 1—Its cholesterol content
  - 2—Its apo(a) content which may make it pro-thrombotic
  - 3—Its content of OxPL

Antisense Drugs Prevent the Translation of a Specific Targeted Protein

- **Transcription**
- **Translation**

**Disease-associated Protein**

- **Traditional Drug**
- **RNase H1**
  - Degrades mRNA
  - No Translation

**No Disease-associated Proteins Produced**

**Antisense Drug**
- (Single stranded, DNA-like)
- (Oligonucleotide)

ExcelMedical

CLINICAL TRIALS
Antisense – The 3rd Wave of Pharmacology

- **1899**: Aspirin
- **1966**: First biologic insulin (pork)
- **1982**: First recombinant biologic
- **1985**: First mAb
- **1989**: First ASO
- **1998**: First ASO: 2nd Generation
- **2013**: Next-Gen ASO

**Small Molecule (<900 Da)**

**Biologics (~6,000-150,000 Da)**

**Antisense (~6,000-10,000 Da)**

**Sources**
Differentiating Pharmacologic Properties of ASOs

• Highly Specific: reduced potential for off-target binding
  • ASOs hybridize specifically to their target mRNA through Watson-Crick base-pair interactions without affecting other mRNAs

• No known drug–drug interactions: metabolized without CYP450 enzymes
  • 90% of drugs are metabolized by CYP450 enzymes, mostly in the liver
  • ASOs are not metabolized via the CYP450 pathway, but rather by endonucleases and exonucleases and are excreted in the urine

• Intact blood brain barriers and placental barriers are impermeable to ASOs
Significant Advantages of GalNAc<sub>3</sub> Conjugate of ISIS 681257

- ASO-GalNAc<sub>3</sub> conjugate approach results in enhanced ASO delivery to hepatocytes versus non-parenchymal cells.
- GalNAc<sub>3</sub>-ASO conjugate is rapidly cleaved to liberate the free ASO.
- Results in equal potency at approximately 1/15<sup>th</sup> of the dose compared to the unconjugated ASOs.
- Greatly reduced dose results in proportionally reduced class effects.
- Flexible dosing - weekly, monthly, quarterly
Mechanism of Action of GalNAc$_3$-Conjugated Antisense Oligonucleotides
Clinical Studies
TG Lowering

• Strength
• CS-7
Clinical Studies
CS-7 APPROACH

An open-label extension study of volanesorsen administered subcutaneously to patients with familial chylomicronemia syndrome (FCS)

- FCS defined by lactescent serum or documentation of fasting TG ≥ 880 mg/dL
- Confirmed for loss of function mutations in Type 1 causing genes (such as LPL, apoC-II, GPIHBP1, or LMF1)
- Fasting TG ≥ 750 mg/dl at screening
Volanesorsen is an Antisense Drug that Prevents the Translation of apo C3

Transcription → Translation

RNase H1 Degrades mRNA → No Translation

No Disease-associated Protein Produced (apo C3)
What's in a Name?

Familial Chylomicronemia Syndrome

• Chylomicronemia syndrome
• Chylomicronemia, familial
• Familial chylomicronemia
• Hyperchylomicronemia familial
• Hyperlipemia idiopathic Burger-Grutz type
• Hyperlipoproteinemia Type I
• Lipase D deficiency
• Lipoprotein lipase deficiency (LPLD)
• Burger-Grutz syndrome

• Endogenous hypertriglyceridemia
• Familial fat-induced hypertriglyceridemia
• Familial hyperchylomicronemia
• Familial LPL deficiency
• Hyperlipidemia Type I (Fredrickson)
• Hyperlipoproteinemia Type IA
• Lipase D deficiency
What is FCS?

- **Background:**
  - Rare autosomal recessive disorder
  - Severely elevated levels of plasma TGs, generally unresponsive to lipid-lowering therapies\(^1\)\(^-\)\(^3\)

- **Clinical expression/risk:**
  - Signs and symptoms:
    - Plasma lactescence and viscosity
    - Lipemia retinalis
    - Abdominal pain
    - Hepatosplenomegaly
    - Eruptive xanthomas
  - Pancreatic insufficiency
  - Recurrent acute pancreatitis/Chronic pancreatitis

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Familial Chylomicronemia Syndrome (FCS): Pathophysiology
Chylomicrons in healthy individual

- Large lipoprotein particles that transport TGs derived from dietary fat\(^1,2\)
- Appear in circulation shortly after a meal; cleared after a few hours\(^3\)
- TGs broken down by LPL to FFAs, which are used by various tissues\(^2\)

Adapted from Braham, Nat Rev Endocrinol, 2015.
Abbreviations: FFA, free fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.
FCS pathophysiology

- Chylomicronemia: pathological persistence of chylomicrons in plasma following a fasting period of 10 to 14 hours\textsuperscript{1,2}
- In FCS, chylomicronemia is caused by inherited defects in chylomicron processing\textsuperscript{2}


Abbreviations: FFA, free fatty acid; TG, triglyceride; VLDL, very low-density lipoprotein.

### Genetics: Known mutations responsible for FCS

<table>
<thead>
<tr>
<th>Gene</th>
<th>Gene product function</th>
<th>Molecular features</th>
<th>% of Monogenic mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPL</td>
<td>Hydrolysis of TGs and peripheral uptake of FFA</td>
<td>Severely reduced or absent LPL enzyme activity</td>
<td>95%</td>
</tr>
<tr>
<td>APOC2</td>
<td>Required cofactor of LPL</td>
<td>Absent or nonfunctional ApoC-II</td>
<td>2.0%</td>
</tr>
<tr>
<td>GPIHBP1</td>
<td>Stabilizes the binding of chylomicrons near LPL</td>
<td>Absent or defective GPI-HBP1</td>
<td>2.0%</td>
</tr>
<tr>
<td>APOA5</td>
<td>Enhancer of LPL activity</td>
<td>Absent or defective apoA-V</td>
<td>0.6%</td>
</tr>
<tr>
<td>LMF1</td>
<td>Chaperone molecule required for proper LPL folding</td>
<td>Absent or defective LMF1</td>
<td>0.4%</td>
</tr>
</tbody>
</table>

Adapted from Brahm, Nat Rev Endocrinol, 2015. Abbreviations: FFA, free fatty acid; LPL, lipoprotein lipase; TG, triglyceride.

Familial Chylomicronemia Syndrome – Clinical/Differential Diagnosis

- Severe HTG (>10 mmol or 880 mg/dL)
- Refractory HTG (not responsive to standard TG therapies)
- Observed > 2x

Exclude

Other Causes of HTG:
- Alcoholism
- Uncontrolled T2DM
- Medications known to HTG*

Potential FCS if any of the following:

- History of acute pancreatitis
- History of recurrent abdominal pain without other explainable cause
- Confirmation by genetic mutation analysis (i.e. genes for LPL, apoCII, GPIHBP1, ApoA5 or LMF1 and/or other genes shown to modulate LPL)

FCS

* e.g. thiazides, beta blockers, estrogen, 2nd generation antipsychotics, Isotretinoin, antiretroviral
FCS Management Goals

**Reduce plasma triglyceride levels**

- Alleviate signs and symptoms/physical manifestations of FCS

**Avoid abdominal pain and recurrent acute pancreatitis**

- Reduce risk of long term consequences

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There are no FDA approved drugs indicated to lower triglycerides in FCS patients

<table>
<thead>
<tr>
<th>Fibrates(^1,2)</th>
<th>Fish oils(^3,4)</th>
<th>Niacin(^5)</th>
<th>Statins(^6,7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increase lipolysis and elimination of TG-rich particles by activating lipoprotein lipase</td>
<td>Increased β-oxidation of DGAT</td>
<td>Inhibits release of free fatty acids from adipose tissue</td>
<td>Block cholesterol synthesis</td>
</tr>
<tr>
<td>Decrease VLDL-C, increase HDL-C</td>
<td><strong>Increase plasma lipoprotein lipase activity</strong>&lt;br&gt;Decrease hepatic TG synthesis</td>
<td><strong>Increases lipoprotein lipase activity</strong>&lt;br&gt;Decreases hepatic synthesis of VLDL, LDL</td>
<td>Increase number of hepatic LDL receptors to enhance uptake and catabolism of LDL</td>
</tr>
<tr>
<td><strong>Increase plasma lipoprotein lipase activity</strong>&lt;br&gt;Decrease hepatic TG synthesis</td>
<td><strong>Decrease hepatic synthesis of VLDL, LDL</strong></td>
<td></td>
<td><strong>Inhibit hepatic synthesis of VLDL and LDL</strong></td>
</tr>
</tbody>
</table>

FCS patients are generally unresponsive to lipid-lowering therapies\(^8\)

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*Proposed; exact mechanisms may not be fully delineated.

Abbreviations: DGAT, diacylglycerol acyltransferase; HDL-C, high-density lipoprotein cholesterol; LDL, low-density lipoprotein; TG, triglyceride; VLDL, very low-density lipoprotein; VLDL-C, very low-density lipoprotein cholesterol.

Management of FCS: Expert Dietary Guidance

Strict Lifelong Dietary Restriction

Sugar Restriction

Extreme low-fat diet (≤15% of energy)

Complete Avoidance of Alcohol

Although extremely difficult to follow, these mainstays of therapy can improve clinical manifestations1,2,3,4,5

- Reduce risk of hepatosplenomegaly
- Reduce abdominal pain
- Reduce risk of xanthomas
- Reduce risk of pancreatitis1,2,3,4,5

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Hypertriglyceridemia is likely the third leading cause of acute pancreatitis.
Pathophysiologic Mechanism of Acute Pancreatitis in FCS Patients

Large Triglyceride-Rich Chylomicrons

Pancreatic lipase leak
Increase FFA production

Impaired pancreatic blood flow

Trypsinogen

Ischemia/Acidosis

Pancreatic Acinar Cell Injury

Acute Pancreatitis

Cardiovascular Inflammation Reduction Trial (CIRT)

A randomized, double-blind, placebo-controlled, event-driven trial of weekly low-dose methotrexate (LDM) in the prevention of cardiovascular events among stable coronary artery disease patients with Type 2 Diabetes or Metabolic Syndrome

- History of MI or multi-vessel CAD
- DM II or Metabolic Syndrome
- NIH Trial
Cardiovascular Inflammation Reduction Trial (CIRT)

hsCRP, a clinical biomarker of inflammation, is commonly used as a method to predict cardiovascular risk

Risk of future heart attack

Risk of future stroke

Relative Risk of MI

Relative Risk of Stroke

Quartile of hsCRP

Quartile of hsCRP

CANTOS

• **Primary Goal**
  
  To determine whether long-term treatment with canakinumab (50 mg, 150 mg or 300 mg subcutaneous every three months) as compared to placebo will reduce rates of recurrent cardiovascular events among stable post-myocardial infarction patients who remain at elevated vascular risk as gauged by increased levels of hsCRP (≥2mg/L) despite usual care, including statin therapy.

• **Positive Trial**: Results in Barcelona 8/17
Clinical Studies
1245.121 HFrEF & 1245.110 HFpEF

Phase III randomized, double-blind study to evaluate efficacy and safety of once daily empagliflozin 10 mg compared to placebo, in patients with heart failure.
Clinical Trials: The path to cure

• Once Cause and Effect have been proved, therapeutics should be optimized

• LDL
• TG
• Lp(a)
• Inflammation
• People want to participate in clinical trials