Therapy of Hypertriglyceridemia: What does the future hold?
Severe Refractory HTG: High Unmet Medical Need

- 3-5000 with LPL or apoC-II deficiency (orphan indications)
- 30-50K with severe refractory HTG (orphan indication approved)
- 25% of patients with HTG have apoA-V polymorphisms

1. Patient
   TG 3700 mg/dL
   Ch 546 mg/dL

2. Healthy
   TG 100 mg/dL
   Ch 200 mg/dL

xanthomas filled with foam cells

Yuan G et al. CMAJ 2007;176:1113-1120
Lipid metabolism with high and normal hepatic TG production

- **TG Production**
  - High
  - Low

- **Very Small VLDL**
  - LPL
  - ApoA5
  - ApoB
  - ApoE

- **Large VLDL**
  - LPL
  - ApoB
  - ApoE
  - ApoC-III

- **Large LDL**
  - ApoB
  - ApoE

- **Remnants**
  - LPL
  - ApoB
  - ApoE
  - ApoC-III

- **Remnant Cholesterol**
  - Non-HDL-C

- **CE**

- **TG**

- **CETP**

- **Renal clearance**

- **HDL**
  - HL
  - sHDL

- **Small LDL**
  - ApoB
  - ApoC-III

- **Very Small LDL**

- **Non-HDL-C**

- **LDL-C**

- **Cholesterol**

- **Triglycerides**
Targeted Therapies for Refractory Hypertriglyceridemia with Genotype based Patient Selection

Lipase enzyme activity is regulated by apolipoprotein

ApoAV peptide  ApoCII peptide

apoC3 ASO  LPL gene therapy

Cholesterol  Triglycerides
Glybera Gene Replacement Therapy for LPL Deficiency

- First Gene replacement therapy being authorized in the occidental world
- Designed for patients with FCS due to loss-of-function LPL gene mutations.
- Not for FCS caused by apoA-5, apoC-2, GPIHPB1, LMF-1 gene mutations.
- Requires genotyping (genetic test)
Glybera Mechanism of Action
Fasting TG Decreased After 12 Weeks of Treatment but Returned to Baseline After 5 Months
Results shown are a mean ± SEM; n=5 (wk-2 and wk+14) or n=3 (wk+52).

p-values: t-test AUC_{24hrs}; wk-2 versus wk+14 and wk+52
Risk Reduction of Definite, Probable Pancreatitis and Abdominal Pain Events

Consistent 56 - 67% risk reduction

- p-values 0.001 - 0.007
ApoC-III inhibits the conversion of VLDL to LDL and causes small dense LDL and low HDL.
### Clinical Experience with 2nd Generation ASOs

- >4000 subjects treated by IV and/or SC administration
- >100 clinical studies
- Multiple therapeutic indications
- >100 patients dosed for >1 year
- Some patients dosed for > 4 years
- Doses as high as 1200 mg tolerated

### Chimeric RNase H ASO Design

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

<table>
<thead>
<tr>
<th>affinity</th>
<th>stability</th>
<th>tolerability</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

### RNase H Terminating Mechanism

- Specific sequence not repeated throughout genome, reducing potential for off-target binding

Phase 2 Open Label Cohort in FCS

- Three patients in ApoCIII<sub>Rx</sub> Study in FCS
  - homozygotes or compound heterozygote for FCS-causing null LPL gene mutations
  - have LPL mass but no or extremely low level (<5%) of LPL activity

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2*</th>
<th>Patient 3*</th>
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</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>Female</td>
<td>Female</td>
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<tr>
<td>Age, yrs</td>
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<td>67</td>
<td>28</td>
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<tr>
<td>BMI, kg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>23.7</td>
<td>29.0</td>
<td>23.4</td>
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<tr>
<td>Genotype</td>
<td>P207L/P207L</td>
<td>P207L/G188E</td>
<td>P207L/P207L</td>
</tr>
</tbody>
</table>

*participated in post-heparin LPL activity measurements
**ISIS-APOCIII<sub>Rx</sub> Treatment Reduced Fasting Plasma ApoC-III and Triglyceride Levels in FCS Patients**

### Fasting ApoC-III Levels

<table>
<thead>
<tr>
<th>Parameter (mg/dL)</th>
<th>Patient No.</th>
<th>Baseline&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Primary Endpoint&lt;sup&gt;†&lt;/sup&gt;</th>
<th>Change from Baseline</th>
<th>% Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td>1</td>
<td>1406</td>
<td>616.5</td>
<td>-789.5</td>
<td>-56.2</td>
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<tr>
<td></td>
<td>2</td>
<td>2083</td>
<td>287.5</td>
<td>-1795.5</td>
<td>-86.2</td>
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<tr>
<td></td>
<td>3</td>
<td>2043</td>
<td>734.5</td>
<td>-1308.5</td>
<td>-64.0</td>
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<tr>
<td>ApoC-III</td>
<td>1</td>
<td>18.9</td>
<td>5.5</td>
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<td>-70.9</td>
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<td></td>
<td>2</td>
<td>35.1</td>
<td>3.4</td>
<td>-31.7</td>
<td>-90.4</td>
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<tr>
<td></td>
<td>3</td>
<td>19.8</td>
<td>3.5</td>
<td>-16.3</td>
<td>-82.5</td>
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</tbody>
</table>
**ISIS-APOCIII**<sub>Rx</sub> Treatment Reduced Fasting Plasma Chylomicron-TG and ApoB-48 Levels in FCS Patients

### Fasting Chylomicron-TG Levels

#### Patient Analysis

<table>
<thead>
<tr>
<th>Parameter (mg/dL)</th>
<th>Patient No.</th>
<th>Baseline</th>
<th>Primary Endpoint&lt;sup&gt;*&lt;/sup&gt;</th>
<th>Change from Baseline</th>
<th>% Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chylomicron-TG</td>
<td>1</td>
<td>1054</td>
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<td>1641</td>
<td>151.5</td>
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<td>1511</td>
<td>622.4</td>
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<td>-58.8</td>
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<tr>
<td>ApoB-48</td>
<td>1</td>
<td>0.98</td>
<td>0.83</td>
<td>-0.15</td>
<td>-14.8</td>
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<tr>
<td></td>
<td>2</td>
<td>1.54</td>
<td>0.27</td>
<td>-1.27</td>
<td>-82.2</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.72</td>
<td>0.53</td>
<td>-0.19</td>
<td>-26.3</td>
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</table>
Non-HDL Cholesterol was also Reduced in Parallel with Triglyceride Levels
High Correlation Between ApoC-III and Triglycerides and Between Chylomicron-TG and Triglycerides
ISIS-APOCIII$_{Rx}$ Safety and Tolerability

- **Drug-related adverse events**
  - No elevations of liver enzymes >3x ULN
  - No abnormalities in renal function
  - No clinically meaningful changes in other laboratory values

- **Tolerability**
  - Generally well tolerated
  - No flu-like symptoms
  - Low incidence of injection site reactions (primarily mild erythema)
Postulated extracellular effects of apoA-V on TG-rich lipoprotein metabolism.

Forte T M et al. J. Lipid Res. 2009;50:S150-S155
Hereditary Defects in apoA-V Cause HTG

- Impaired synthesis of apoA-V (1131T>C variant) linked to HTG in 25% of patients
- 50K participants in 27 studies

APOA1/C3/A4/A5 “gene cluster” & dyslipidemia

![Gene Cluster Diagram]

Triglyceride (n=45730)

Percentage mean difference (95% CI)

Genotype

<table>
<thead>
<tr>
<th></th>
<th>T/T*</th>
<th>T/C</th>
<th>C/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglyceride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (n=38266)</td>
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<td></td>
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</tr>
<tr>
<td>LDL cholesterol (n=26878)</td>
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</tr>
<tr>
<td>Apolipoprotein Al (n=9819)</td>
<td></td>
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<tr>
<td>Apolipoprotein B (n=10589)</td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

Sarwar et al., Lancet 2010
APOA5 Causal for both Hypertriglyceridemia and Premature Cardiovascular Disease

**APOA5 Association with Lipid levels in Patients with HTG**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Triglyceride (n=45730)</th>
<th>HDL cholesterol (n=38266)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/T*</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>T/C</td>
<td>10%</td>
<td>20%</td>
</tr>
<tr>
<td>C/C</td>
<td>-10%</td>
<td>-20%</td>
</tr>
</tbody>
</table>

**APOA5 Association in Patients with Coronary Heart Disease**

- Genetically-raised triglyceride† (20842 cases/35206 controls)
- Risk ratio (95% CI)

50K participants in 27 studies

21K cases, 35K controls in 39 studies
Apo-A5 has a High Impact at Low Concentrations

- Attractive template for anti-HTG peptidomimetics
- Apo-A5 physiologically active at low concentrations

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>Effect on TG levels</th>
<th>Plasma concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoC-III</td>
<td>↑</td>
<td>3.4 μM</td>
</tr>
<tr>
<td>apoC-II</td>
<td>↓</td>
<td>34.7 μM</td>
</tr>
<tr>
<td>apoA-V</td>
<td>↓</td>
<td>0.0038 μM</td>
</tr>
</tbody>
</table>

- Apo-a5 knockout mice have 4x higher TG (*Science*, 2001)
- Apo-A5 gene expression lowers TG in normal mice by ~ 66%
- Plasma concentration is 10,000-fold less than apo-C2 or Apo-Al
**Technology: AV-peptide to Stimulate Lipase Activity**

- AV-peptides bind to VLDL, lipase & heparan sulfate (acts as a “reservoir”)

- Lead-peptide (AV-H/K) can boosts lipase activity 8x

### Rate of TG Hydrolysis

![Graph showing rate of TG hydrolysis for different treatments](image)

- VLDL
- AV199-232
- AV201-232
- AV205-232
- AV-H/K

**Cells, tissues**
Apo-CII Peptidomimetic Structure

- Apo-C2 peptidomimetic is 2 alpha helices joined together – 36 aa

- Helix1 is a high affinity peptide for lipoproteins - previously described, 18A

- Helix2 derived from apo-CII LPL activating domain

- More effective than full length Apo C2

- Increased ABCA1 cholesterol efflux
Effective in both Apo-C2 Deficient and General HTG Patients

- Addition of Apo-C2 ex vivo was highly efficacious in Apo-C2 deficient patients
- Was also efficacious in HTG population – over 50 subjects tested
- Data suggest an enhanced efficacy in the presence of LPL
Apo-C2 modulates TG and Cholesterol Levels in Apo-E Knockout Mice

- Apo-E Knockout mice were given Apo-C2 bolus and followed for 4 hours
- Consistent with in vitro activities, there was a decrease in both TG and Cholesterol
DGAT Catalyzes TG Synthesis

glycerol-3-phosphate → lysophosphatidate → phosphatidate → monoacylglycerol → diacylglycerol → triacylglycerol

Glycerol Phosphate Pathway
Monoacylglycerol Pathway

DGAT
Role of Acyl CoA: diacylglycerol acyltransferase 1 (DGAT1) in the Absorption of Fat

- DGAT1 catalyzes final step in triglyceride synthesis
- DGAT1 is expressed in gut > adipose, liver, skeletal muscle, heart
Inhibition of DGAT2 Decreases Hepatic Triglyceride (TG) Accretion and Secretion

4 hrs with 0.4 mM [3H]oleate

Li et al, ATVB 2015
DGAT Inhibition as a Treatment for Obesity

- Pharmaceutical companies have DGAT inhibitor programs
- Some data from clinical trials with DGAT1 inhibitor are available but it does not look all that good: diarrhea
- Why so different from mice?:
  - A rare DGAT1 mutation in human is associated with congenital diarrheal disorder (very severe): kids die within 6 months
- DGAT2 inhibitor has been developed

Harris et al. JCI 2012
FXR Agonism for Hypertriglycerideridemia and NASH

- HDL declines reflect upregulation of reverse cholesterol transport
- TG declines from SREBP-1c downregulation
- LDL increase from CETP block? Increased LPL activity?
Improvement in NAS components

Steatosis

Percent of subjects improved

- Placebo: 38%
- OCA: 61%

Inflammation

Percent of subjects improved

- Placebo: 35%
- OCA: 53%

Ballooning

Percent of subjects improved

- Placebo: 31%
- OCA: 46%

Change in score

- Placebo: -0.4
- OCA: -0.8

- Placebo: -0.2
- OCA: -0.5

- Placebo: -0.2
- OCA: -0.5

Serum lipids

A Treasure Trove of Information for Lipoprotein Biology

Omics and Emerging TG-Lowering Therapies

Potential targets for gene replacement therapy:
- LPL, apoC2, ApoA-5, GPIHBP1

Potential targets for anti-sense therapy:
- apoB, apoC-III, DGAT2, MicroRNAs, Aptamers

Cell Pathways:
Peptide linker technologies

metabolic pathways:
DGAT-2inh, MTPI, FXR agonism

Peptide-based mimetics:
- ApoC2, apoE, ApoC5
- Monoclonal Ab-ANGLPT 3