IONIS-ANGPTL3-LRx, an antisense inhibitor to angiopoietin-like protein 3 [ANGPTL3] reduces plasma ANGPTL3 and lipids in healthy volunteers with elevated triglycerides

Teresa A. Brandt¹, Li-Jung Tai¹, Joseph L. Witztum², Steven G. Hughes¹, Eunju Hurh³, Brad McEvoy¹, Rosie Yu¹, Andres Digenio³, Richard Lee¹, Mark Graham¹, Rosanne Crooke¹, Sotirios Tsimikas¹,4

¹Ionis Pharmaceuticals, Inc., Carlsbad, California, ²University of California, San Diego, Department of Medicine, Division of Endocrinology & Metabolism, ³Akcea Therapeutics, Cambridge, Massachusetts, ⁴University of California, San Diego, Department of Medicine, Division of Cardiovascular Diseases
Disclosures:
Teresa A. Brandt, Li-Jung Tai, Steven G. Hughes, Eunju Hurh, Brad McEvoy, Rosie Yu, Andres Digenio, Richard Lee, Mark Graham, Rosanne Crooke, and Sotirios Tsimikas are employees of Ionis Pharmaceuticals or Akcea Therapeutics

Joseph L. Witztum is a consultant to Ionis Pharmaceuticals, Intercept, CymaBay and Prometheus.

Joseph L. Witztum, Sotirios Tsimikas are co-inventors and receive royalties from patents owned by the University of California San Diego on oxidation-specific antibodies
Angiopoietin-Like 3 (ANGPTL3) is a Genetically Validated Lipid and Metabolic Target in Humans

• Genome-wide association and exome sequencing studies have identified ANGPTL3 genetic variations that are associated with very low plasma LDL-C, HDL-C and TG\textsuperscript{1-3}

• ANGPTL3 complete loss-of-function mutations result in familial combined hypolipidemia (FHBL2), which is manifested by a reduction of all lipoproteins, except Lp(a)\textsuperscript{4}

• Loss of function of ANGPTL3 results in increased lipoprotein lipase and endothelial lipase activities, enhanced insulin sensitivity and decreased serum FFAs\textsuperscript{5}

Effect of ANGPTL3 LOF Mutations on Lipid Phenotypes

Pooled analysis in 115 subjects with FHBL2 with 13 different mutations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>% Difference</th>
<th>p-value</th>
<th>% Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL-C</td>
<td>-8.6%</td>
<td>0.007</td>
<td>-67.2%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>TG</td>
<td>-21.1%</td>
<td>0.005</td>
<td>-71.2%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>HDL-C</td>
<td>-16.8%</td>
<td>p&lt;0.001</td>
<td>-39.0%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoB</td>
<td>-7.2%</td>
<td>0.008</td>
<td>-48.4%</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>ApoA1</td>
<td>-13.1%</td>
<td>0.001</td>
<td>-95.1%</td>
<td>p&lt;0.001</td>
</tr>
</tbody>
</table>

Other clinical characteristics of homozygotes
- No increase in prevalence of fatty liver compared to controls
- No increased atherosclerosis or other manifestations of CVD
- Improved insulin sensitivity, lower plasma glucose, and lower incidence of T2DM

Minicoci I, et al. JLR, 2013
Inhibition of ANGPTL3 May Have Multiple Beneficial Effects in Lipoprotein Metabolism

RNA-Targeted Antisense Drugs Block the Translation of ANGPTL3 Protein

DNA  mRNA  Disease-associated Protein

Transcription  Translation  RNase H1 Degrades mRNA=No Translation

Transcription  Translation  No Disease-associated Proteins Produced

Antisense Drug (Single stranded, DNA-like)

The Antisense Drug-Receptor Interaction Occurs by Classical Watson-Crick Hybridization

Antisense Oligonucleotide RNA Target

- Unmodified DNA and RNA do not make good drugs due to insufficient stability and distribution, and rapid degradation in plasma
- Chemical modification of ribose backbone can convert oligonucleotides to therapeutic agents
- Specificity and optimal binding usually requires a length of 15-20 bases
Significant Advances in Medicinal Chemistry
Improve Potency and Tolerability

1st Gen
P-S
↑10X

Gen 2/2+
MOE Gapmer Design
↑10X

Gen 2.5
cEt Gapmer Design
↑10X

LICA
GalNac Design
↑10X
IONIS-ANGPTL3\textsubscript{Rx} and IONIS-ANGPTL3-L\textsubscript{Rx} Sequences and Gapmer Technology- GaINAc Gen 2+

IONIS-ANGPTL3-L\textsubscript{Rx} contains GaINAc and 6 PO

**Chimera / Gapmer**

- RNase H1 Substrate
- **MOE**
- **Deoxy**
- **MOE**

- **GGAC**
- **TTGC**
- **AGTA**
- **TCGC**

**affinity**

**stability**

**tolerability**

**ANGPTL3\textsubscript{Rx} Gen 2+**

- GsGsAsCsAsTsTsGsCsAsGsTsAsAsTsCsGsCsA

**ANGPTL3-L\textsubscript{Rx} GaINAc Generation 2+**

- 5’-THA-
- GalNac

- MOE

- PS

- PO

- GsGoAoCoAoTsTsGsCsAsGsTsAsAsToCoGsCsA
Comparison of Dose-Response Curves of IONIS-ANGPTL3\textsubscript{Rx} vs. IONIS-ANGPTL3-L\textsubscript{Rx} Following 6 Weeks of SC Administration in Humans

Note, the study for IONIS-ANGPTL3\textsubscript{Rx} had two additional loading doses (on Days 3 and 5) compared to IONIS-ANGPTL3-L\textsubscript{Rx}.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>IONIS-ANGPTL3\textsubscript{Rx}</th>
<th>IONIS-ANGPTL3-L\textsubscript{Rx}</th>
</tr>
</thead>
<tbody>
<tr>
<td>$ED_{50}$ (mg)</td>
<td>$210 \pm 1.1$</td>
<td>$10.4 \pm 1.2$</td>
</tr>
<tr>
<td>$\gamma$</td>
<td>$-2.57 \pm 0.46$</td>
<td>$-1.11 \pm 0.20$</td>
</tr>
</tbody>
</table>

~21X higher potency
Pre-Clinical Data: ANGPTL3Rx Significantly Reduces Plasma Cholesterol and Plasma and Liver TGs in Ldlr -/- Mice

**Angptl3 Plasma Protein**
- Control ASO (50 mg/kg/wk)
- ANGPTL3 ASO (50 mg/kg/wk)
- ANGPTL3 ASO (12.5 mg/kg/wk)

* Indicates significantly different ($p<0.05$) when compared to control ASO treatment.
Pre-Clinical Data: ANGPTL3Rx Reduces Liver Triglyceride Accumulation in a Diet-Induced Obesity Mouse Model

Liver TG

Lipogenic Gene Expression

* Denotes significantly different when compared to control ASO

ᶲ Denotes significantly different when compared to MTP ASO
PRE-Clinical Data: Murine-Specific ANGPTL3\textsubscript{Rx} ASO Significantly Reduced the Progression of Atherosclerosis in \textit{Ldlr} \textsuperscript{-/-} Mouse Model

* Indicates significantly different ($p<0.05$) when compared to control ASO treatment

16 weeks post treatment
Background: Phase I Trial
IONIS-ANGPTL3$_{Rx}$ Reduces ANGPTL3 Protein in Healthy Volunteers

- Triglycerides reduced by up to 63% (group means up to 49%)
- Total cholesterol reduced by up to 46% (group means up to 28%)
- Larger reductions observed in subjects with higher baseline lipid levels

N=8 for Placebo, N=6 for each IONIS-ANGPTL3$_{Rx}$ Dose Group

*p<0.05  **p<0.01  ***p<0.001
**Study Design Phase 1/2a Clinical Study**
**IONIS-ANGPTL3-L\textsubscript{Rx} - SAD/MAD**

<table>
<thead>
<tr>
<th></th>
<th>Single Ascending Dose (N=12)</th>
<th>Multiple Ascending Dose (N=32)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment</strong></td>
<td>1 SC dose</td>
<td>6 once-weekly SC doses</td>
</tr>
<tr>
<td><strong>Subjects</strong> (active:placebo)</td>
<td>N = 4 per dose cohort (3:1)</td>
<td>N = 8 per dose cohort (6:2)</td>
</tr>
<tr>
<td><strong>Dose levels</strong> (dose volume)</td>
<td>20, 40 or 80 mg (0.2, 0.4, 0.8 mL per dose)</td>
<td>10 mg, 20 mg, 40 mg or 60 mg (0.1, 0.2, 0.4, 0.6 mL per dose)</td>
</tr>
<tr>
<td><strong>Post-treatment</strong></td>
<td>30-90 days</td>
<td>13 weeks</td>
</tr>
</tbody>
</table>

**Inclusion Criteria:**
- Healthy volunteers age 18-65, BMI <35 kg/m\textsuperscript{2}
- MAD cohorts required TG >150 mg/dL and LDL-C >70 mg/dL

**Key Endpoints:**
- Safety and tolerability
- Change in fasting LDL-C, HDL-C, VLDL-C, TC, TG, non-HDL-C, apoA-I, apoB, LDL:HDL ratio, TC:HDL ratio, Lp(a), apoC-III, and ANGPTL3
- PK of ANGPTL3-L\textsubscript{Rx}
RESULTS: IONIS ANGPTL3-LRx 80 mg Single Dose
Mean % Change in Plasma ANGPTL3 Levels

![Graph showing change in plasma ANGPTL3 levels over study days](image-url)
RESULTS: IONIS ANGPTL3-L_{Rx} 80 mg Single Dose
Mean % Change in Lipid Parameters
RESULTS: Baseline Characteristics of the MAD Cohorts
IONIS-ANGPTL3-LRx Phase I/2a Trial

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Placebo (N=8)</th>
<th>10 mg IONIS-ANGPTL3-LRx (N=6)</th>
<th>20 mg IONIS-ANGPTL3-LRx (N=6)</th>
<th>40 mg IONIS-ANGPTL3-LRx (N=6)</th>
<th>60 mg IONIS-ANGPTL3-LRx (N=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANGPTL3 (ng/mL)</td>
<td>126.1 (32.5)</td>
<td>84.5 (23.5)</td>
<td>96.8 (19.3)</td>
<td>112.4 (7.8)</td>
<td>109.7 (38.3)</td>
</tr>
<tr>
<td>Total Cholesterol (mg/dL)</td>
<td>216 (12)</td>
<td>217 (35)</td>
<td>216 (32)</td>
<td>230 (31)</td>
<td>206 (48)</td>
</tr>
<tr>
<td>LDL-C (mg/dL)</td>
<td>132 (16)</td>
<td>133 (41)</td>
<td>141 (29)</td>
<td>154 (32)</td>
<td>128 (42)</td>
</tr>
<tr>
<td>TG (mg/dL)</td>
<td>201 (36)</td>
<td>212 (107)</td>
<td>196 (50)</td>
<td>212 (62)</td>
<td>168 (60)</td>
</tr>
<tr>
<td>VLDL-C (mg/dL)</td>
<td>38 (10)</td>
<td>39 (20)</td>
<td>38 (12)</td>
<td>38 (11)</td>
<td>33 (13)</td>
</tr>
<tr>
<td>HDL-C (mg/dL)</td>
<td>46 (11)</td>
<td>46 (16)</td>
<td>37 (7)</td>
<td>38 (5)</td>
<td>45 (10)</td>
</tr>
<tr>
<td>ApoB (mg/dL)</td>
<td>108 (13)</td>
<td>107 (28)</td>
<td>120 (15)</td>
<td>120 (13)</td>
<td>103 (39)</td>
</tr>
<tr>
<td>ApoA1 (mg/dL)</td>
<td>146 (18)</td>
<td>149 (32)</td>
<td>131 (15)</td>
<td>129 (10)</td>
<td>137 (16)</td>
</tr>
<tr>
<td>ApoC-III (mg/dL)</td>
<td>12.6 (2.9)</td>
<td>11.2 (4.6)</td>
<td>9.7 (1.9)</td>
<td>11.0 (2.8)</td>
<td>9.7 (3.1)</td>
</tr>
<tr>
<td>Lp(a) (nmol/L)</td>
<td>35 (23)</td>
<td>70 (72)</td>
<td>32 (53)</td>
<td>20 (28)</td>
<td>10 (14)</td>
</tr>
<tr>
<td>Non-HDL-C (mg/dL)</td>
<td>170 (19)</td>
<td>172 (36)</td>
<td>180 (30)</td>
<td>192 (27)</td>
<td>161 (50)</td>
</tr>
</tbody>
</table>
RESULTS: IONIS-ANGPTL3-L_{Rx} Phase I/2a Trial

MAD Cohort – Mean % Change in ANGPTL3 Levels

- Placebo
- IONIS-ANGPTL3-L_{Rx} 20mg
- IONIS-ANGPTL3-L_{Rx} 60mg
- IONIS-ANGPTL3-L_{Rx} 10mg
- IONIS-ANGPTL3-L_{Rx} 40mg

*p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001

Fasting ANGPTL3 (ng/mL)

Mean % Change (+/- SEM)

Study Day

12 8 15 22 29 36 37 43/ET 50 64 92 127

treatment period
RESULTS: IONIS-ANGPTL3-L_{Rx} MAD Cohort
Mean % Change in Lipid Levels (Day 37)

![Graph showing mean % change in lipid levels from baseline with SEM error bars.](image)

* *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001
IONIS-ANGPTL3-L$_{Rx}$ Phase 1/2a Study Safety and Tolerability

- No injection site reactions
- No flu-like symptoms
- No reductions in platelet count
- No drug-related SAEs
- No study discontinuations due to AEs
CONCLUSIONS

- IONIS-ANGPLT3-L_{Rx} reduced plasma levels of ANGPTL3 up to 83% in healthy volunteers with elevated triglyceride levels.

- Significant mean reductions were noted in TGs (-66%), apoC-III (-68%), LDL-C (-35%), total cholesterol (-36%), HDL-C (-25%) and non-HDL-C (-40%).

- Among all known therapies that lower TG levels, this is associated not only with reduced levels of LDL-C but total apoB as well.

- No safety concerns were identified related to target reduction or drug administration.

- IONIS-ANGPLT3-L_{Rx} is a promising candidate for patients with poorly controlled LDL-C, elevated TG and possibly in patients with hepatic steatosis or NASH.
Acknowledgements

Ionis/Akcea:
Stan Crooke
Richard Geary
Brett Monia
Scott Henry
Anthony Scozzari
John Su
Brenda Baker
Walter Singleton
Erika Paz
Laurence Gamelin
LJ Shen
Paula Soteropoulos
Louis O’Dea