How are we going to intervene in elevated Lp(a)?
From PCSK9 to mRNA inhibition

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Cardiovascular Franchise Leader
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ISA 2015
May 25 2015
Lp(a) a risk factor in patients on long term statin therapy in the current era

LIPID
JUPITER
AIM HIGH
Plasma Lipoprotein(a) Concentration Predicts Future Coronary and Cardiovascular Events in Patients With Stable Coronary Heart Disease
Paul J. Nestel, Elizabeth H. Barnes, Andrew M. Tonkin, John Simes, Marion Fournier, Harvey D. White, David M. Colquhoun, Stefan Blankenberg and David R. Sullivan

Arterioscler Thromb Vasc Biol. 2013;33:2902-2908; originally published online October 3, 2013;
doi: 10.1161/ATVBAHA.113.302479

Lipoprotein(a) Concentrations, Rosuvastatin Therapy, and Residual Vascular Risk: An Analysis From the JUPITER Trial (Justification for the Use of Statins in Prevention: An Intervention Trial Evaluating Rosuvastatin)
Amit V. Khera, Brendan M. Everett, Michael P. Caulfield, Feras M. Hantash, Jay Wohlgemuth, Paul M Ridker and Samia Mora

Circulation. 2014;129:635-642; originally published online November 17, 2013;
Lp(a) remains a predictor of CVD events in patients with normal Lp(a) levels (13 mg/dl) and LDL-C of 54 mg/dl

Albers et al
JACC 2013

Figure 2
Time to First Cardiovascular Event for Statin Plus Placebo Arm by Baseline Lp(a) Quartile
Potential Clinical Indications for Lp(a) Lowering

- Apheresis for elevated Lp(a)
- CVD with recurrent events
- Refractory angina
- Calcific aortic valve stenosis
- FH with elevated Lp(a)
- End stage renal disease
- Post ACS
- Pediatric stroke
- Secondary prevention
- Primary prevention
## Therapeutic Agents Affecting Lp(a) Levels

<table>
<thead>
<tr>
<th>Increase</th>
<th>Decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Statins</td>
<td>Clinically available</td>
</tr>
<tr>
<td>Low fat diets</td>
<td>LDL apheresis</td>
</tr>
<tr>
<td>Garlic supplements</td>
<td>Niacin</td>
</tr>
<tr>
<td>Growth hormone</td>
<td>Fish oil</td>
</tr>
<tr>
<td></td>
<td>Mipomersen</td>
</tr>
<tr>
<td></td>
<td>IL-6 antagonists</td>
</tr>
<tr>
<td></td>
<td>? Aspirin</td>
</tr>
<tr>
<td></td>
<td>? Lomitapide</td>
</tr>
<tr>
<td></td>
<td>Investigational</td>
</tr>
<tr>
<td></td>
<td>PCSK9 Inhibitors</td>
</tr>
<tr>
<td></td>
<td>CETP Inhibitors</td>
</tr>
<tr>
<td></td>
<td>ASO to apo(a)</td>
</tr>
<tr>
<td></td>
<td>Other</td>
</tr>
<tr>
<td></td>
<td>Thyroid analogues</td>
</tr>
<tr>
<td></td>
<td>Oral estrogen/tamoxifen</td>
</tr>
<tr>
<td></td>
<td>Anabolic steroids</td>
</tr>
<tr>
<td></td>
<td>Neomycin</td>
</tr>
<tr>
<td></td>
<td>N-acetylcysteine</td>
</tr>
<tr>
<td></td>
<td>L-carnitine</td>
</tr>
</tbody>
</table>

**Increase**

- Statins
- Low fat diets
- Garlic supplements
- Growth hormone

**Decrease**

### Clinically available

- LDL apheresis
- Niacin
- Fish oil
- Mipomersen
- IL-6 antagonists
- ? Aspirin
- ? Lomitapide

### Investigational

- PCSK9 Inhibitors
- CETP Inhibitors
- ASO to apo(a)

### Other

- Thyroid analogues
- Oral estrogen/tamoxifen
- Anabolic steroids
- Neomycin
- N-acetylcysteine
- L-carnitine
Extended-Release Nicotinic Acid: Data from Pivotal Placebo-Controlled Studies

Change from Baseline

HDL-C

LDL-C

Lp(a)

TG

Extended-Release Nicotinic Acid: Data from Pivotal Placebo-Controlled Studies

Change from Baseline

HDL-C

LDL-C

Lp(a)

TG
Effect of diet, drug therapy and apheresis on LDL-C
The effect of Lp(a) lowering by lipid apheresis in very high-risk patients with recurrent events

120 patients with CAD and Lp(a) > 95th percentile

Stage 1: Lipid-lowering medication until maximal tolerated doses (mean 5.6 years)

Stage 2: Combined lipid apheresis + lipid-lowering medication (mean 5.0 years)

- Lp(a) reduced by 73%, LDL-C reduced by 65%
- Major coronary events reduced by 86%

LDL-C in stage 1 ≤ 100 mg dL⁻¹
  - true LDL-C: −5 mg dL⁻¹
  - major events −89%

LDL-C in stage 1 > 100 mg dL⁻¹
  - true LDL-C: −61 mg dL⁻¹
  - major events −85%

Similar reduction in events
MACE during lipid-lowering medication alone and during combined lipid-lowering medication and lipid apheresis

Apheresis for Isolated elevated Lp(a) >60 mg/dL reduces MACE

Effect of alirocumab on Lp(a)

A

Baseline Lp(a) change from baseline (LOCF)

<table>
<thead>
<tr>
<th></th>
<th>≤30 mg/dl</th>
<th>&gt;30 mg/dl</th>
<th>≤50 mg/dl</th>
<th>&gt;50 mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients</td>
<td>n=74</td>
<td>n=102</td>
<td>n=43</td>
<td>n=51</td>
</tr>
</tbody>
</table>

Median percentage Lp(a) change from baseline (LOCF)

<table>
<thead>
<tr>
<th></th>
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<th>&gt;30 mg/dl</th>
<th>≤50 mg/dl</th>
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<td>n=74</td>
<td>n=102</td>
<td>n=43</td>
<td>n=51</td>
</tr>
</tbody>
</table>

B

Baseline Lp(a) change from baseline (mg/dL; LOCF)

<table>
<thead>
<tr>
<th></th>
<th>≤30 mg/dl</th>
<th>&gt;30 mg/dl</th>
<th>≤50 mg/dl</th>
<th>&gt;50 mg/dl</th>
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<td>n=74</td>
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<td>n=51</td>
</tr>
</tbody>
</table>

*P<0.0001 vs. placebo
†P<0.001 vs. placebo

*P<0.001 vs. placebo

Pooled placebo
Pooled alirocumab 150 mg Q2W
Lp(a) change from baseline (%) vs. LDL-C change from baseline in alirocumab treatment group

R-square: 0.0463
Spearman’s correlation coefficient: 0.2236
p=0.0298
Reduction in Lipoprotein(a) With PCSK9 Monoclonal Antibody Evolocumab

- Evolocumab Q2W
  - 70 mg: -13.8%
  - 105 mg: -25.2%
  - 140 mg: -29.5%

- Evolocumab Q4W
  - 280 mg: -18.7%
  - 350 mg: -21.3%
  - 420 mg: -24.5%
Weak correlation between change in Lp(a) and change LDL-C and apoB With Evolocumab

A
Observations: 1082
Spearman Correlation Coefficient: 0.5134

B
Observations: 1087
Spearman Correlation Coefficient: 0.5203
What is the mechanism of Lp(a) reduction by PCSK9 antibodies?

- Unknown
- Unlikely to be decreased apo(a) synthesis
- PCSK9 may regulate an Lp(a) receptor or directly prevent clearance of apo(a) and PCSK9 antibodies interfere with this process
# CETP-Inhibition - Anicetrapib

## Lipid Parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>LS Mean Percent (95% CI) Placebo-Adjusted Change from Baseline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 24</td>
</tr>
<tr>
<td>Non-HDL-C</td>
<td>-31.7* (-33.6, -29.8)</td>
</tr>
<tr>
<td>Apo B</td>
<td>-21.0* (-22.7, -19.3)</td>
</tr>
<tr>
<td>Apo A-1</td>
<td>44.7* (42.8, 46.5)</td>
</tr>
<tr>
<td>TC</td>
<td>13.7* (12.0, 15.3)</td>
</tr>
<tr>
<td>TG</td>
<td>-6.8 (-9.9, -3.9)</td>
</tr>
<tr>
<td>Lp(a)</td>
<td>-36.4 (-40.7, -32.3)</td>
</tr>
<tr>
<td>ApoE</td>
<td>29.2* (24.7, 33.7)</td>
</tr>
</tbody>
</table>

*p<0.001; means for all variables except for triglycerides, lipoprotein(a), for which medians are shown*
Antisense Technology Reduces Disease Causing Protein Levels by Targeting mRNA

Traditional Small Molecule Drugs
Inhibitors or Agonists of proteins

Biologics
Inhibitors or Mimics of proteins

DISEASE

Antisense Oligonucleotide
Inhibition of RNA function
(no production of disease causing protein)
Antisense Mechanism of Action
RNase H1 Terminating Mechanism

Specific sequence not repeated throughout genome, reducing potential for off-target binding
Second Generation 2'-MOE Gapmer Chemistry

*Increased Affinity, Stability, Tolerability and Simple Formulation*

**Chimeric RNaseH ASO Design**

- ↑ affinity
- ↑ stability
- ↑ tolerability

**RNase H Substrate**

**2’-O-methoxyethyl (MOE)**

**Clinical Experience**
- Kynamro approved by the FDA
- >6000 subjects dosed (IV/SC)
- >100 clinical studies
- Multiple therapeutic indications
- >140 patients dosed for >1 year
- Some patients dosed for 4 years
- Doses as high as 1200 mg tolerated
# Evolution of Antisense Chemistries

<table>
<thead>
<tr>
<th>Chemistry Attributes</th>
<th>1st Generation Phosphorothioate (PS)</th>
<th>2nd Generation MOE Gapmer</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Adds stability</td>
<td>✓ Increases potency</td>
<td></td>
</tr>
<tr>
<td>✓ Improves distribution to tissues</td>
<td>✓ Increases stability</td>
<td></td>
</tr>
<tr>
<td></td>
<td>✓ Reduces non-specific toxicities</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Potency</th>
<th>1200 to 3500 mg/week</th>
<th>~100 to 400 mg/week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose Frequency</td>
<td>Daily to 3x/week</td>
<td>Weekly to monthly</td>
</tr>
<tr>
<td>Cost of Therapy</td>
<td>Between branded small molecules &amp; antibodies</td>
<td>Competitive with upper end of small molecules</td>
</tr>
<tr>
<td>Routes of Administration</td>
<td>I.V., enema, intravitreal</td>
<td>Sub Q, I.V., inhalation, topical, intrathecal</td>
</tr>
</tbody>
</table>
Asialoglycoprotein Receptor (ASGPR) – Identification and Characterization

- ASGPR is abundantly expressed by mammalian hepatocytes
- 500,000 – 1 million copies per cell
- Requires calcium for ligand binding
- Functional receptor is comprised of two subunits, HL-1 and HL-2
- Maintains serum glycoprotein levels by endocytosing desialylated glycoproteins with terminal galactose or GalNAc residues
- Specificity for galactose and N-acetylgalactosamine (GalNAc, GN) terminated oligosaccharides
  - Highest affinity for tri- and tetra-antennary ligands
  - Affinity for GalNAc ligands with low nM affinity
  - GalNAc conjugated ASOs improve potency for liver targets by 7-10x in mice
Insights into Lp(a) synthesis: Reduction in Lp(a) levels with antisense oligonucleotides (ASO) to apoB or apo(a) in mouse models

Mipomersen, an Antisense Oligonucleotide to Apolipoprotein B-100, Reduces Lipoprotein(a) in Various Populations with Hypercholesterolemia: Results of 4 Phase III Trials
Mipomersen reduces Lp(a) levels - Individual Phase 3 Trials
Long-term efficacy and safety of mipomersen in patients with familial hypercholesterolemia: 2-year interim results of an open-label extension
Sustained Reductions in LDL-C, Apo B, and Lp(a) During Long-Term Mipomersen Treatment

Mean % Change (+/-95% CI)

<table>
<thead>
<tr>
<th>Week</th>
<th>LDL-C</th>
<th>Apo B</th>
<th>Lp(a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>26</td>
<td>130</td>
<td>111</td>
<td>57</td>
</tr>
<tr>
<td>52</td>
<td>104</td>
<td>156</td>
<td>30</td>
</tr>
<tr>
<td>104</td>
<td>208</td>
<td>234</td>
<td>17</td>
</tr>
</tbody>
</table>

N=141
Mipomersen reduces Lp(a) levels

- 4 Phase 3 Trials: Modest correlation between change in LDL-C vs. change in Lp(a)

Tsimikas et al ACC 2013
MACE Rate was Significantly Reduced (~7 fold) in FH Patients Treated with Mipomersen for ≥1 Year

<table>
<thead>
<tr>
<th></th>
<th>Before Mipomersen</th>
<th>During Mipomersen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient-years</td>
<td>207</td>
<td>211</td>
</tr>
<tr>
<td>Observation Time, years *</td>
<td>1.99 (0.01)</td>
<td>2.03 (0.73)</td>
</tr>
<tr>
<td>Total Events</td>
<td>146</td>
<td>12</td>
</tr>
<tr>
<td>MI</td>
<td>39</td>
<td>2</td>
</tr>
<tr>
<td>PCI/CABG</td>
<td>99</td>
<td>6</td>
</tr>
<tr>
<td>Unstable Angina</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Stroke</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Incidence, n (%)</td>
<td>64 (61.5%)</td>
<td>9 (8.7%)</td>
</tr>
<tr>
<td><strong>MACE Rate †</strong></td>
<td><strong>25.7</strong></td>
<td><strong>3.6</strong></td>
</tr>
</tbody>
</table>

* Values presented are the mean (SD).
† The number of patients with at least one event divided by the total follow-up time in months (x 1000).
Significant Reduction in MACE Incidence in FH Patients Treated with Mipomersen for ≥1 Year

OR [95% CI]: 0.035 [0.009 - 0.144]
P<0.0001
ISIS-APO(a)_{Rx} is Designed to Lower Plasma Lp(a)
Second Generation Antisense Drug

2‘ Methoxyethyl Phosphorothioate Oligonucleotide (2’ MOE Gapmer)

ISIS-APO(a)_{Rx} targets the splice site of exon 24/25 of apo(a) mRNA, corresponding to a Kringle-IV_{2} repeat

It does not bind to or reduce hepatic expression of plasminogen mRNA
ISIS-APO(a)$_{Rx}$

Mechanism of Action in Reducing Plasma Lp(a)

Tsimikas et al, Lancet 2015 In Press
Objectives
- Evaluate the safety & tolerability of ISIS-APO(a)_{Rx} in healthy volunteers
- Evaluate effect on Lp(a) levels 2 weeks after the last dose

Exploratory Objectives
- Change in lipid profile and apolipoprotein B-100 2 weeks after the last dose
- Change in OxPL/apoB levels 2 weeks after the last dose
- Impact of apo(a) isoforms on treatment response
## Baseline Characteristics of Multiple Ascending Dose Groups

<table>
<thead>
<tr>
<th></th>
<th>Placebo (n=6)</th>
<th>100 mg (n=8)</th>
<th>200 mg (n=9)</th>
<th>300 mg (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender (M:F)</strong></td>
<td>6:0</td>
<td>8:0</td>
<td>9:0</td>
<td>8:0</td>
</tr>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>31</td>
<td>41</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>24.2</td>
<td>27.0</td>
<td>24.1</td>
<td>26.8</td>
</tr>
<tr>
<td><strong>Lipids &amp; Lipoproteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lp(a) (nmol/L)</strong></td>
<td>152±83</td>
<td>92±74</td>
<td>82±67</td>
<td>107±76</td>
</tr>
<tr>
<td><strong>Total Cholesterol (mg/dL)</strong></td>
<td>194±23.5</td>
<td>199±29.4</td>
<td>196±32.1</td>
<td>200±31.1</td>
</tr>
<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>46±6.6</td>
<td>49±7.5</td>
<td>52±14.7</td>
<td>51±13.6</td>
</tr>
<tr>
<td><strong>ApoB (mg/dL)</strong></td>
<td>89±15</td>
<td>95±19</td>
<td>86±23</td>
<td>89±17</td>
</tr>
<tr>
<td><strong>LDL-C (mg/dL)</strong></td>
<td>121±22.3</td>
<td>131±27.2</td>
<td>116±35.5</td>
<td>131±22.7</td>
</tr>
<tr>
<td><strong>Triglycerides (mg/dL)</strong></td>
<td>137±52.9</td>
<td>96±29.4</td>
<td>151±161.4</td>
<td>94±39.2</td>
</tr>
<tr>
<td><strong>OxPL-apoB-100 (nmol/L)</strong></td>
<td>11.0±6.1</td>
<td>6.7±4.5</td>
<td>8.8±5.6</td>
<td>9.1±4.9</td>
</tr>
<tr>
<td><strong>Major apo(a) isoform (#KIV repeats)</strong></td>
<td>21.5±4.5</td>
<td>20.1±3.1</td>
<td>23.0±5.0</td>
<td>20.6±4.0</td>
</tr>
</tbody>
</table>

ITT population, values presented are the mean.

* Entry Criterion of Lp(a) ≥ 25 nmol/L (~10 mg/dL) for MAD cohorts
†Excludes 2 subjects who received < 3 doses of Study Drug
ISIS-APO(a)\textsubscript{Rx} Phase I Trial

Mean percent change in Lp(a) over time by treatment group in the multiple-dose cohort

Tsimikas et al, Lancet 2015 In Press

**p<0.01
***p<0.001
ISIS-APO(a)\textsubscript{Rx} Phase I Trial

Relationship of plasma ISIS-APO(a)\textsubscript{Rx} trough concentrations and mean percent change in Lp(a), OxPL-apoB and OxPL-apo(a)

Tsimikas et al, Lancet 2015 In Press
Mean % change in Lp(a), OxPL-apoB and OxPL-apo(a) is independent of baseline Lp(a) levels Day 36 in the 300mg multiple-dose group

Tsimikas et al, Lancet 2015 In Press
**ISIS-APO(a)\textsubscript{Rx} Phase I Trial**

*Mean percent change in Lp(a), OxPL-apoB, OxPL-apo(a), total cholesterol, LDL-C, apoB, HDL-C, and triglycerides*

![Graph showing mean percent change from baseline to Day 36 for various parameters under different treatments: Placebo, ISIS-APO(a)\textsubscript{Rx} 200 mg, ISIS-APO(a)\textsubscript{Rx} 100 mg, and ISIS-APO(a)\textsubscript{Rx} 300 mg.]

- Placebo
- ISIS-APO(a)\textsubscript{Rx} 200 mg
- ISIS-APO(a)\textsubscript{Rx} 100 mg
- ISIS-APO(a)\textsubscript{Rx} 300 mg

**Statistical Significance**
- *p<0.05
- **p<0.01
- ***p<0.001

Tsimikas et al, Lancet 2015 In Press

P = NS
Phase 2: ISIS 494372-CS3
ClinicalTrials.gov Identifier: NCT02160899

“Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of ISIS-APO(a)$_{\text{Rx}}$ in Patients With High Lipoprotein(a)”
### ISIS-APO(a)\textsubscript{Rx} (CS3)

**Phase 2 Study in Patients with Lp(a) >50 mg/dL**

- **Randomized, double-blind, placebo-controlled, intra-patient, dose-titration study**

- **2 Cohorts**
  - Cohort A: High Lp(a) (>50 mg/dl); 1:1 (active : placebo)
  - Cohort B: Very high Lp(a) (>175 mg/dl); 4:1 (active : placebo)
  - Intra-patient dose titration

- **Objectives**
  - Evaluate activity of ISIS-APO(a)\textsubscript{Rx} in lowering Lp(a)
  - Evaluate the safety & tolerability of ISIS-APO(a)\textsubscript{Rx}

- Phase 2 initiation 1H 2014

<table>
<thead>
<tr>
<th>Cohorts</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>50</td>
</tr>
<tr>
<td>B</td>
<td>10</td>
</tr>
</tbody>
</table>

**Treatment Period**
- 12 Weeks
- 12 weekly s.c. injections

- **Post-Treatment f/u Period**
- 16 weeks

- 28 days
- Screening

**Treatment Period**
- 100 mg
- 200 mg
- 300 mg
Phase 1: ISIS-APO(a)-L$_{Rx}$
ClinicalTrials.gov Identifier: NCT02414594

“Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of ISIS APO(a)-L$_{Rx}$ in Healthy Volunteers With Elevated Lipoprotein(a)”
Objectives

- Evaluate the safety & tolerability of ISIS 681257 in healthy volunteers
- Evaluate effect on Lp(a) levels 2 weeks after the last dose
- Evaluate PK effects of single and multiple doses of ISIS 681257

Exploratory Objectives

- Change in lipid profile and apolipoprotein B-100 2 weeks after the last dose
- Change in OxPL/apoB levels 2 weeks after the last dose
- Impact of apo(a) isoforms on treatment response