Novel high-throughput ELISAs to measure apoC-III on apoB-100, apoA-I and LDL: Reduction in lipoprotein-associated apoC-III levels with valsaranesol (ISIS 304801) or ISIS-APOC-IIIra antisense therapy to apoC-III in a Phase 2 randomized trial

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1) Apo-C III can be detected directly on apoB-100, Lp(a) and apoA-I by immunocapture from plasma.

2) These ELISA techniques are adaptable to high-throughput methodology.

3) Use of ELISAs significantly reduces apo-C III on apoB-100, Lp(a) and LDL in hyper-triglyceridemic patient populations.

4) These assays may be used to understand the cardiovascular effects of triglyceride lowering therapies.

5) Whether these predictive cardiac effects will produce clear clinical endpoints will have to be determined in future studies.

References


Disclosures

II and III are co-inventors and receive royalties from Isis Pharmaceuticals for the lipoprotein-associated apoC-III antibodies. A patent has been applied for US/CA on lipoprotein-associated apoC-III antibodies in lipIDS, US/CA 15/660,579. A second patent application for developing a dual appointment at UCSD and Gilead Sciences. J.L.W. is a consultant for two Pharmaceutics, Intercept and Prometheus. V.A. and O’D are employees of Isis Pharmaceuticals. The other co-authors have no conflicts of interest.

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Results - Baseline Patient Characteristics

Table 1: Results – Baseline Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Placebo (N=28)</th>
<th>100 mg (N=30)</th>
<th>200 mg (N=30)</th>
<th>300 mg (N=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>29.1 ± 4.4</td>
<td>28.5 ± 5.1</td>
<td>28.4 ± 5.1</td>
<td>28.5 ± 4.8</td>
</tr>
<tr>
<td>Triglyceride, mg/dL</td>
<td>150 ± 110</td>
<td>150 ± 110</td>
<td>150 ± 110</td>
<td>150 ± 110</td>
</tr>
<tr>
<td>LDL-C</td>
<td>95.4 ± 25.3</td>
<td>93.5 ± 25.2</td>
<td>92.1 ± 25.0</td>
<td>91.1 ± 25.1</td>
</tr>
<tr>
<td>HDL-C</td>
<td>40.9 ± 9.2</td>
<td>40.4 ± 9.1</td>
<td>39.6 ± 9.0</td>
<td>39.2 ± 9.0</td>
</tr>
</tbody>
</table>

### Results - Baseline Characteristics of Subgroups

- **LDL-C**: The reduction in LDL-C from baseline was significantly greater with both 100 mg and 200 mg compared to placebo (p<0.05).
- **HDL-C**: The increase in HDL-C from baseline was significantly greater with 200 mg compared to 100 mg and placebo (p<0.05).
- **Triglyceride**: The reduction in triglyceride levels was similar across all doses.

**Aims**

- The CVR risk of circulating plasma apo-C III is highly dependent on its association with lipoproteins. For example, LDL or HDL containing apo-C III predict CVR, whereas LDL or HDL without apo-C III does not, even after adjustment for triglycerides. Currently established methods to detect apo-C III on LDL or HDL utilize labor-intensive methods that can only be done on small numbers of samples and are not easily translatable to large-scale outcomes or clinical applications. Therefore, our goal was to develop high-throughput sandwich ELISAS to detect apo-C III on individual lipoproteins, directly from plasma, as a tool to test their relationship to CVR risk and effectiveness of apo-C III-lowering agents. These agents include antisenesce dioligosaccharides (ApoC) and drugs that indirectly affect apo-C III, such as fibrates, fish oil or other triglyceride lowering agents.

**Methods**

- A 225 dBp periglomerular antibody was readily detected in placebo at 0.016 mg/ml, whereas there was no substantial difference in the APOC-I levels of subjects on different diets.
- For APOC-I levels, a p<0.05 was observed for the regression of apo-A-I levels with HDL-C at 150 mg/dL.
- For APOC-II levels, the effect was significant for the regression of apo-A-I levels with LDL-C at 150 mg/dL.

**Results**

- APOC-II levels were significantly lower in the placebo group compared to the treatment group, indicating a decrease in triglyceride levels.
- APOC-III levels were significantly lower in the placebo group compared to the treatment group, indicating a decrease in triglyceride levels.

**Preliminary Analysis**

- Preliminary analysis of the data indicated that the APOC-I levels were significantly lower in the placebo group compared to the treatment group, indicating a decrease in triglyceride levels.
- Preliminary analysis of the data indicated that the APOC-II levels were significantly lower in the placebo group compared to the treatment group, indicating a decrease in triglyceride levels.
- Preliminary analysis of the data indicated that the APOC-III levels were significantly lower in the placebo group compared to the treatment group, indicating a decrease in triglyceride levels.