CHARACTERIZATION OF THYROID HORMONE RECEPTOR (THR) AGONISTS FOR THE TREATMENT OF NON-ALCOHOLIC STEATOHEPATITIS (NASH) BY QUANTIFICATION OF GENE TRANSCRIPTION IN HUMAN HEPATOCYTES

Xuan (Susan) G. Luong1, Sarah K. Stevens1, Andreas Jekel1, Tse-I Lin2, Kusum Gupta3, Dinah Misner3, Sushmita Chanda1, Sucheta Mukherjee1, Jyanwei Liu1, Caroline Williams1, Antitsa Stoycheva1, Lawrence M. Blatt1, Leonid N. Beigelman1, Julian A. Symons1, Pierre Raboisson2, Dave McGowan2, Koen Vandyck2, and Jerome Deval1

1Aligos Therapeutics, Inc., South San Francisco, CA; 2Aligos Belgium BV, Leuven, Belgium

E-mail: sxuong@aligos.com

Abstract #1665

1. Background
Thyroid hormones are important modulators of metabolic activity in mammals and alter cholesterol and fatty acid levels through activation of the thyroid hormone receptor (THR). Currently, there are several THR agonists in clinical trials for the treatment of NASH that have exhibited potential to reduce liver fat and restore liver function. In this study, we compared the ability of THR agonism-based NASH treatment candidates, GC-1, MGL-3196, and VK2809, to modulate the expression of genes related to cholesterol and fatty acid biosynthesis and metabolism in vitro using human hepatic cells and in vivo using the rat model.

2. Methods
Activation of THR in Huh-7 cells and primary human hepatocytes (PHH) was measured by changes in CPT1A and THRSP RNA levels, respectively, using RT-qPCR. The most and least potent compounds as characterized by cell-based assays, triiodothyronine (T3) and MGL-3196, respectively, were evaluated for in vivo efficacy in rats fed high-fat diets (HFD). Serum total and low-density lipoprotein cholesterol (LDL-C) levels were measured and RT-qPCR for liver Dio1 and Me1 was performed.

3. Results

Figure 1 CPT1A dose-response curves in treated Huh-7 cells

Figure 2 THRSP dose-response curves in treated PHH

Table 1 Average EC50 values from in vitro gene expression assays

<table>
<thead>
<tr>
<th></th>
<th>T3</th>
<th>GC-1</th>
<th>MGL-3196</th>
<th>VK2809A</th>
<th>VK2809</th>
</tr>
</thead>
<tbody>
<tr>
<td>Huh-7 EC50 (nM)</td>
<td>0.3 ± 0.03 (n = 22)</td>
<td>1.3 ± 0.2 (n = 5)</td>
<td>303.1 ± 50.9 (n = 17)</td>
<td>8.3 ± 2.2 (n = 5)</td>
<td>589.1 ± 120.1 (n = 5)</td>
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<tr>
<td>Mean ± SEM</td>
<td>1.0 ± 0.6 (n = 4)</td>
<td>2.7 ± 1.6 (n = 4)</td>
<td>216.2 ± 197.5 (n = 3)</td>
<td>14.8 ± 10.7 (n = 4)</td>
<td>18.7 ± 8.9 (n = 3)</td>
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- **0.5 mg/kg T3 significantly decreased total cholesterol and LDL-C levels**
- **MGL-3196 lowered serum lipid levels in a concentration-dependent manner, but significant decreases were only observed at doses ≥ 1.5 mg/kg and the compound was much less potent than T3**
- **Treatment with T3 and MGL-3196 resulted in pronounced increases in liver Dio1 and Me1 transcript levels that mirror decreases in serum lipids**

4. Conclusions
We have implemented a strategy to rank the efficacy of THR agonists by quantifying changes in the transcription of genes that lead to metabolic alterations, an effect that is directly downstream of THR binding and activation. By using human-derived hepatic cells, this method provides more biologically relevant data compared to biochemical or non-hepatocyte-based screening assays. Our observations in vitro were confirmed in a HFD-fed rat model, where treatment with THR agonists resulted in significant, dose-dependent increases in the liver gene expression that correlate well to reduction in lipid levels. These data taken together support using the quantification of gene expression as a measurement of THR agonist efficacy.

References

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