Molecular, cellular, and pharmacological characterization of beta-selective partial agonists of human thyroid hormone receptor for the treatment of nonalcoholic steatohepatitis

David McGowan¹, Koen Vandycq², Sarah Stevens¹, Xuan Luong¹, Andreas Jekle¹, Tse-I Lin¹, Kusum Gupta¹, Dinah Misner¹, Sushmita Chanda¹, Sucheta Mukherjee¹, Jyanei Liu¹, Caroline Williams¹, Antitza Stoycheva³, Leo Beigelman¹, Julian A. Symons¹, and Pierre Raboisson², and Jerome Deval¹*

¹Aligos Therapeutics, Inc., South San Francisco, CA, ²Aligos Belgium BV, Leuven, Belgium, *Corresponding author: jdeval@aligos.com

Abstract # 2604

Background
Nonalcoholic steatohepatitis (NASH) is characterized by liver inflammation and damage caused by a buildup of fat in the liver. Although no drugs have been approved for the treatment of NASH, thyroid hormone receptor β (THR-β) agonists have demonstrated potential to reduce liver fat, restore liver functions, and possibly reverse fibrosis.¹ Here we present the preclinical development of THR-β partial agonists, defined as a THR-β agonist with limited activation of the THR-α isomere, thereby potentially maximizing efficacy and minimizing safety risk.

Effect of a Single Dose of ALG-136 in SD Rats on a High Fat Diet (HFD)

Rat High Fat Diet Model: Sprague-Dawley rats were fed a high fat diet (D12109C; 20% fat, 1.25% cholesterol, 0.5% cholic acid) for two weeks, followed by a single oral dose of 0.5 or 1.5 mg/kg of ALG-136. Liver and heart tissues were extracted, and gene expression levels were determined by qRT-PCR.

In Vitro Activation of THR-α/β

Biochemical Assay
• TR-FRET thyroid receptor beta coactivator assay

THR-β/THR-α Reporter Cell-based Assay
• Luciferase THR/RAR assay in HEK 293T cells

Huh-7 qPCR Cell-based Assay
• CPTα3a (Carnitine palmitoyltransferase 3A), a key mitochondrial enzyme involved in fatty acid metabolism

In Vitro ADME Properties of ALG-136

adME Assays
Mouse / Rat / Human Liver Microsomes EC₅₀ (min)
99.30 / 99.36 / 99.61

CYP 2C8 / CYP 2B6 (nM) / (µM)
69 / 49
HCR Inhibition IC₅₀ > 10 µM
P 450, 7A1, 9A4, 1A2, 3A4, 2C8, 2C9

Kinetic Solubility (µM)
FaSSIF (pH 6.5)
462
FedFaSSIF (pH 5.0)
543
HCl (pH 2)
9.4

GSH Adduct Negative

Potency and Selectivity of Selective Partial Agonists

<table>
<thead>
<tr>
<th>Compound</th>
<th>EC₅₀a (nM)</th>
<th>EC₅₀b (nM)</th>
<th>EC₅₀c (nM)</th>
<th>EC₅₀d (nM)</th>
<th>a/b selectivity</th>
<th>b/c selectivity</th>
<th>CPTα3a IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALG-114</td>
<td>2.39 (4.6)</td>
<td>0.02 (0.0)</td>
<td>0.01 (0.0)</td>
<td>0.01 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.13 (0.0)</td>
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<tr>
<td>ALG-102</td>
<td>0.01 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.05 (0.0)</td>
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<tr>
<td>ALG-163</td>
<td>0.006 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.05 (0.0)</td>
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<tr>
<td>ALG-131</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.05 (0.0)</td>
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<tr>
<td>ALG-139</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.05 (0.0)</td>
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<tr>
<td>ALG-136</td>
<td>0.005 (0.0)</td>
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<td>0.005 (0.0)</td>
<td>0.005 (0.0)</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>0.05 (0.0)</td>
</tr>
</tbody>
</table>

References:

Conclusions
Evaluation of novel structures revealed THR-β selective partial agonists culminating in the selection of ALG-136 that has reduced THR-α activation in both the biochemical and cellular assays vs. other THR-β agonists currently in development. ALG-136 was stable in rodent and human liver microsomes, has a favorable ADME profile and demonstrated high plasma and liver exposure in rat. After single oral administration, low doses of ALG-136 reduced total cholesterol in a rat high fat diet efficacy model. Dose-dependent increases in the liver expression of Dio2 confirmed target engagement and coincided with reduction in cholesterol. Mgl expression in heart tissue remained relatively unchanged. These findings coupled with limited decreases in T₃, T₄ plasma levels enabled the proof of concept that THR-β partial agonists could potentially be useful in the treatment of NASH.

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