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Abstract # 2149

Preclinical Development of ALG-055009 as a Potent and Selective Thyroid Hormone Receptor Beta Agonist for the Treatment of NASH

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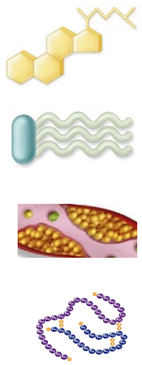
ALIGOS THERAPEUTICS

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 [1-4]. Here we present the preclinical development of a second-generation THR-β agonist with improved potency and favorable selectivity.

Beneficial Effects of THR-β Agonists on NAFLD/NASH



↓ Cholesterol
- **↓ synthesis** (↓ HMGCoA Reductase)
- **↑ catabolism** (↑ Chol. 7$^{\alpha}$ hydrolase = Cyp7A)
- **↑ liver uptake** (↑ HDL receptor SR-B1 expression, ↑ LDL receptor expression)

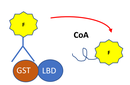
↓ Triglycerides (and fatty acids)
- **↓ synthesis** (↓ Sterol Regulatory Element Binding Transcription Factor-1 = SREBP1c also ↓VLDL assembly)
- **↑ catabolism** (↑ Mitochondrial O_2 consumption & Thermogenesis via ACC, FAS, spot14 etc…)
- **↑ liver uptake** (↑ HDL receptor SR-B1 expression, ↑ LDL receptor expression)

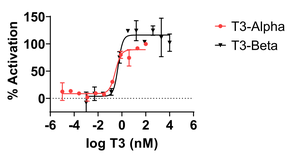
↓ Atherosclerosis plaques
- ↓ LDL and HDL cholesterol
- ↓ ApoA1 lipoprotein
- ↓ Lipoprotein(a) Lp(a)

↓ Insulin resistance
- Effect on glycogenolysis and glycogenesis

In Vitro Activation of THR-β and –α by ALG-055009 and other Agonists

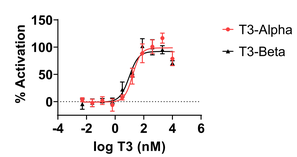
- **THR-β /THR-α Coactivator Recruitment Assay**
  - TR-FRET assay (biochemical)
  - GST-LBD + Fluor-SRC2 peptide





Compound	EC_{50} α (nM)	EC_{50} β (nM)	α/β selectivity
T_3	0.28	0.37	0.8
Eprotirome	0.24	0.63	0.4
GC-1	0.35	0.61	0.6
MGL-3196	**833**	**64**	**13**
VK-2809 parent	**21**	**8.6**	**2.4**
ALG-055009	**360**	**63**	**5.8**

- **THR-β /THR-α Reporter Cell-based Assay**
  - luciferase THR/RXR assay
  - HEK 293T cells



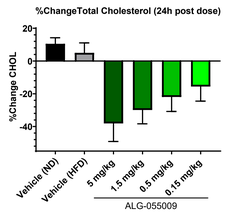
Compound	EC_{50} α (nM)	EC_{50} β (nM)	α/β selectivity
T_3	13.4	11.6	1.2
Eprotirome	30.4	29.5	1
GC-1	8.9	4.2	2.1
MGL-3196	**5,900**	**1,760**	**3.4**
VK-2809 parent	**223**	**220**	**1**
ALG-055009	**196**	**51**	**3.8**

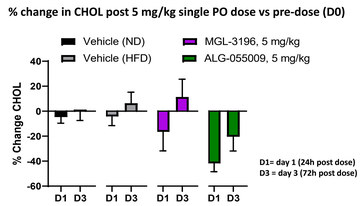
- **Biochemical assay: THR-β EC_{50} of ALG-055009 = 0.063 µM, THR-α/β selectivity = 5.8x**
- **Cell-based reporter assay: THR-β EC_{50} of ALG-055009 = 0.051 µM, THR-α/β selectivity = 3.8x**
  - **35x more potent against THR-β than MGL-3196, and 3.6x more selective than VK-2809**

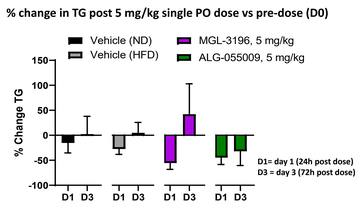
Effect of a Single Dose of ALG-055009 in Rats Fed with High Fat Diet (HFD)

HFD rat PD model:
- Sprague-Dawley rats
- 2-week diet
- 1.5% cholesterol
- 0.5% cholic acid

Single P.O. dose (n=6)
Group 1: Normal diet (ND), vehicle
Group 2: High fat diet (HFD), vehicle
Group 3: HCD, ALG-055009, 5 mg/kg
Group 4: HCD, ALG-055009, 1.5 mg/kg
Group 5: HCD, ALG-055009, 0.5 mg/kg
Group 6: HCD, ALG-055009, 0.15 mg/kg

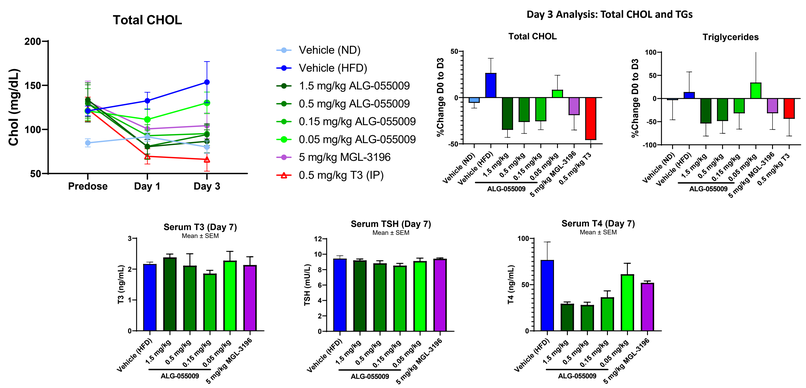






- **Dose-dependent reduction in total cholesterol (CHOL) following single dose of ALG-055009 from 5 to 0.15 mg/kg**
- **Marked reduction of CHOL and triglycerides (TG) at 5 mg/kg ALG-055009**
- **ALG-055009 has a more pronounced effect than MGL-3196 on CHOL after 24h (D1) and 72h (D3)**
- **Sustained reduction of CHOL and TG noted 3 days post dosing with ALG-055009**

Repeat Dosing of ALG-055009 in HFD Rat Model



- **Marked and dose-dependent reduction of total cholesterol and TG with ALG-055009 with a minimum effective dose of 0.15 mg/kg**
- **No dose related impact on T3 and TSH, indicating no effect on hypothalamic-pituitary-thyroid axis**
- **Reduction in T4, also seen with MGL-3196, has been linked to THRβ-induced increase in liver deiodinase 1 expression [5]**

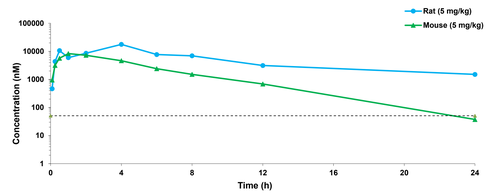
ALG-055009 In Vitro ADME/Tox Overview

ADME/Tox Assays	ALG-055009
Mouse/Rat/Dog/Monkey/Human Liver Microsome $t_{1/2}$ (min)	All >60
Plasma Protein Binding	96.1% to 98.7%
$P_{app\ A->B}$ (10^{-6} cm/s) Efflux Ratio	2.2 6.2
CYP Inhibition 1A2/2B6/2C8/2C9/2C19/2D6/3A4	IC_{50} > 10 µM all
hERG Inhibition	IC_{50} > 10 µM
Nav1.5 and Cav1.2 Inhibition	IC_{50} > 10 µM
Off-Target Screening @10 µM (% binding)	No hits
Kinase Panel	No hits
In vitro genotoxicity assays	Negative

- **ALG-055009 has demonstrated good ADME properties and a clean off-target profile:**
  - **No in vitro reactive metabolite liability**
  - **No CYP or off-target kinase/receptor liability**

Oral PK Profile of ALG-055009 in Mice and Rats

Oral PK Parameters at 5 mg/kg	Mouse	Rat
Plasma C_{max} (nM)	8,341	17,779
Plasma AUC_{inf} (nM.h)	42,277	141,388
Plasma $AUC_{0\text{-}last}$ (nM.h)	38,125	146,680
Liver $AUC_{0\text{-}last}$ (nM.h)	519,410	1,574,177
Liver/Plasma AUC Ratio	13.6	10.7



- **ALG-055009 had low clearance and demonstrated high oral bioavailability**
- **Exposure in liver is >10-fold higher than in plasma in mice and rats**

Conclusions

ALG-055009 has enhanced potency and selectivity vs. other THR-β compounds currently in development and a favorable in vitro safety and ADME profile. In a rodent diet induced efficacy model, low doses of ALG-055009 markedly reduced cholesterol and triglycerides. Taken together, these results warrant further development of ALG-055009 as a potential treatment for NASH.

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