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Abstract # 2604

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

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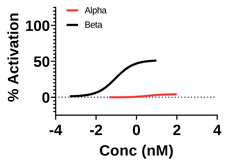
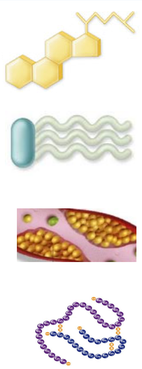
Background

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 THR-β partial agonists, defined as a THR-β agonist with limited activation of the THR-α isoform, thereby potentially maximizing efficacy and minimizing safety risk.

Selective Partial Agonist



Beneficial Effects of THR-β Agonists on NAFLD/NASH



↓ Cholesterol
- ↓ **synthesis** (↓ HMGCoA Reductase)
- ↑ **catabolism** (↑ Chol. 7ª 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)

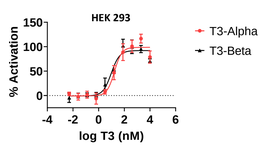
↓ Insulin resistance
- Effect on glycogenolysis and glycogenesis

In Vitro Activation of THR-α/β

Biochemical Assay
- TR-FRET thyroid receptor beta coactivator assay

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

Huh-7 qPCR Cell-based Assay
- *CPT1a* (Carnitine palmitoyl transferase 1A), a key mitochondrial enzyme involved in fatty acid metabolism



Compound	Biochemical EC_{50} α (nM)	Biochemical EC_{50} β (nM)	Biochemical α/β selectivity	HEK 293T Cells EC_{50} α (nM)	HEK 293T Cells EC_{50} β (nM)	HEK 293T Cells α/β selectivity	Gene Expression *CPT1a* EC_{50} (nM)
T_3	0.3	0.4	0.8	14.2	11.5	1.2	0.3
MGL-3196	934	73	12.8	5,927	2,366	2.5	303
VK-2809 parent	26	10	2.5	366	269	1	8

Potency and Selectivity of Selective Partial Agonists

		ALG-114	ALG-152	ALG-163	ALG-131	ALG-189	ALG-136
Biochemical	EC_{50} α [μM]	2.99 (9%)*	0.548 (5%)	0.298 (5%)	>50 (9%)	>50 (7%)	0.396 (10%)
	EC_{50} β [μM]	0.063 (17%)	0.062 (25%)	0.068 (23%)	0.050 (22%)	0.034 (31%)	0.040 (24%)
	Ratio (α/β)	48	9	4	>100	>100	10
HEK 293	EC_{50} α [μM]	>10 (2%)	7.1 (19%)	>10 (10%)	>10 (3%)	10 (19%)	>0.518 (30%)
	EC_{50} β [μM]	0.153 (63%)	0.103 (50%)	0.260 (84%)	0.088 (68%)	0.098 (64%)	0.095 (67%)
	Ratio (α/β)	>65	54	38	>100	102	>5
CPT1a Huh-7 EC_{50} (μM)		1.037	0.368	0.198	0.182	0.144	0.126

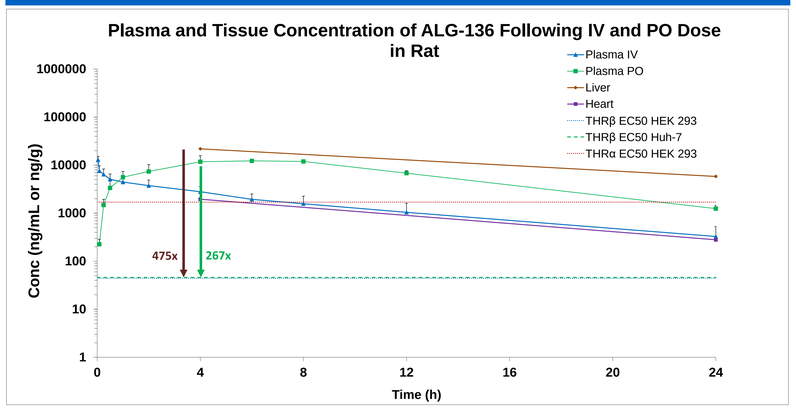
*In parentheses, % activation relative to T3 (100% in both the biochemical and HEK 293 assays)

- Structural variations led to a selective, potent partial agonists.
- ALG-136 demonstrated:
  - ➢Partial agonism in both the biochemical and cell-based assays, with reduced % activation.
  - ➢Highest potency in the gene expression assay, demonstrating the best target engagement *in vitro*.
  - ➢An acceptable *in vitro* ADME profile.

In Vitro ADME Properties of ALG-136

ADME Assays		ALG-136
Mouse / Rat / Human Liver Microsome $t_{1/2}$ (min)		All > 60
Mouse / Rat / Human Plasma Protein Binding (% bound)		99.30 / >99.96 / 99.61
$P_{app\ A->B}$ (10^{-6} cm/s) / Efflux Ratio		1.7 / 5.1
CYP 2C8 / 2C9 Inhibition (% at 10 μM)		69 / 49
hERG Inhibition		IC_{50} > 10 μM
Kinetic Solubility (μM)	PBS (7.4)	132
	FaSSIF (pH 6.5)	462
	FeSSIF (pH 5)	543
	HCl (pH 2)	9.4
GSH Adduct		Negative

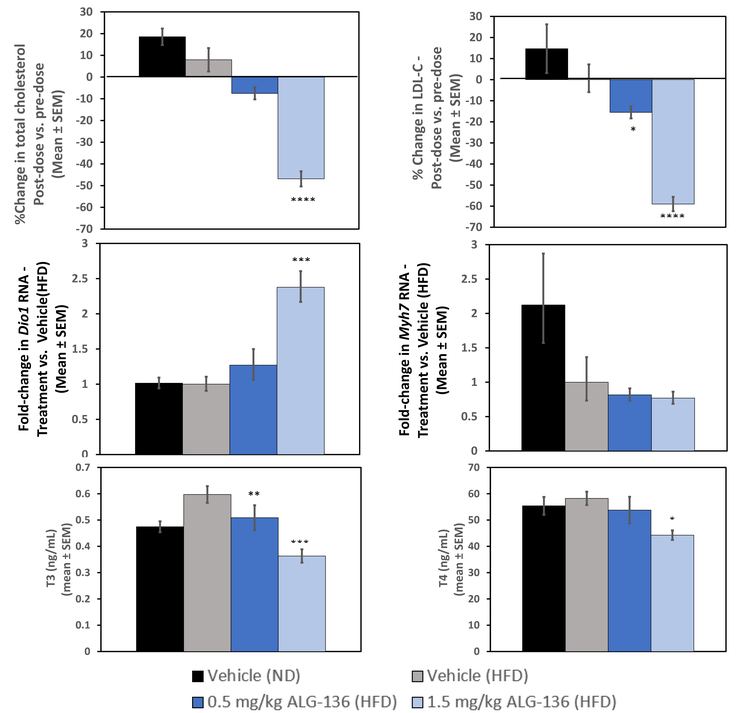
Rat PK Profile of ALG-136



- IV (1 mg/kg) and PO (5 mg/kg), 80% PEG400 in water
- Plasma C_{max} > 250x over THR-β HEK and Huh7 assays EC_{50}
- Liver C_{max} > 475x over the THR-β HEK and Huh7 assays EC_{50}
- Rat PK: L/P = 4.7, L/H = 21

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.



Dio1; Iodothyronine Deiodinase 1. *Myh7;* beta (β)-myosin heavy chain (MHCβ)

Conclusion

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 cell-based 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 *Dio1* confirmed target engagement and coincided with reduction in cholesterol. *Myh7* 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.

References:

1) Pharmacotherapy for NASH: Current and emerging. Konerman MA, Jones JC, Harrison SA. J Hepatol. 2018, 68(2), 362. doi: 10.1016/j.jhep.2017.10.015. 2) Non-alcoholic steatohepatitis: emerging molecular targets and therapeutic strategies. Musso G, Cassader M, Gambino R. Nat Rev Drug Discov 2016, 15(4), 249. doi: 10.1038/nrd.2015.3. 3) Thyroid hormone analogues and derivatives: Actions in fatty liver. Coppola M, Glinni D, Moreno M, Cioffi F, Silvestri E, Goglia F. World J Hepatol 2014, 6(3), 114. doi: 10.4254/wjh.v6.i3.114. 4) Lipid lowering in healthy volunteers treated with multiple doses of MGL-3196, a liver-targeted thyroid hormone receptor-β agonist. Taub R, Chiang E, Chabot-Blanchet M, Kelly MJ, Reeves RA, Guertin MC, Tardif JC. Atherosclerosis. 2013, 230(2), 373. doi: 10.1016/j.atherosclerosis.2013.07.056. 5) Targeting thyroid hormone receptor-beta agonists to the liver reduces cholesterol and triglycerides and improves the therapeutic index. Erion MD, Cable EE, Ito BR, Jiang H, Fujitaki JM, Finn PD, Zhang BH, Hou J, Boyer SH, van Poelje PD, Linemeyer DL. Proc Natl Acad Sci USA. 2007, 104(39), 15490.

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