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

ALIGOS THERAPEUTICS

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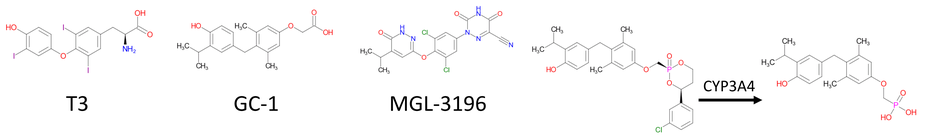
Abstract #1665

## 1. Background

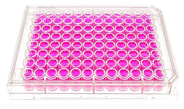
Thyroid hormones are important modulators of metabolic activity in mammals and alter cholesterol and fatty acid levels through activation of the thyroid hormone receptor (THR). Currently, there are several THR agonists in clinical trials for the treatment of NASH that have exhibited potential to reduce 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

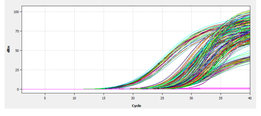
Activation of THR in Huh-7 cells and primary human hepatocytes (PHH) was measured by changes in *CPT1A* and *THRSP* RNA levels, respectively, using RT-qPCR. The most and least potent compounds as characterized by cell-based assays, triiodothyronine (T3) and MGL-3196, respectively, were evaluated for *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.

Huh-7 and PHH cell culture



cDNA

TaqMan RT-qPCR



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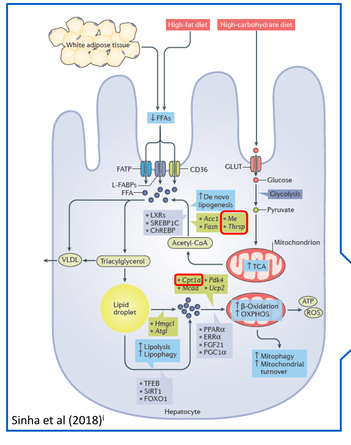


Sinha et al (2018)[i]

HFD fed rat model
- Male Sprague-Dawley rats
- Fed HFD for 2 weeks (D12109C)
- Single-dose study
- Sample blood and liver tissue

Liver THR-regulated genes
- ↑ *CPT1A*: carnitine palmitoyltransferase 1A
- ↑ *THRSP*: thyroid hormone responsive
- ↑ *Dio1*: iodothyronine deiodinase 1
- ↑ *Me1*: malic enzyme

## 3. Results

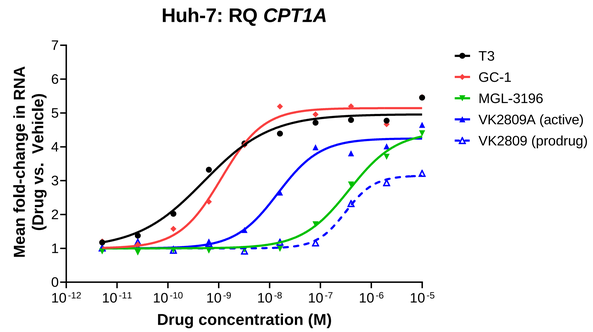


**Figure 1 *CPT1A* dose-response curves in treated Huh-7 cells**

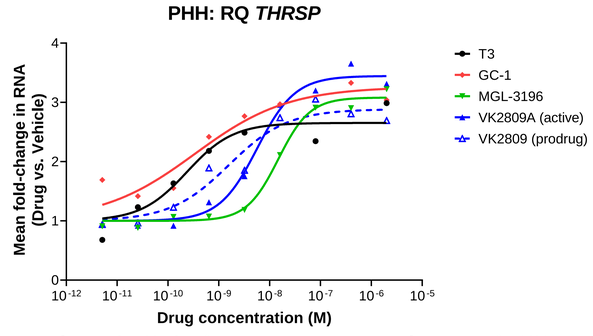


**Figure 2 *THRSP* dose-response curves in treated PHH**

| | T3 | GC-1 | MGL-3169 | VK2809A | VK2809 |
|---|---|---|---|---|---|
| **Huh-7 $EC_{50}$ (nM)** Mean ± SEM | 0.3 ± 0.03 (n = 22) | 1.3 ± 0.2 (n = 5) | 303.1 ± 50.9 (n = 17) | 8.3 ± 2.2 (n = 5) | 589.1 ± 120.1 (n = 5) |
| **PHH $EC_{50}$ (nM)** Mean ± SEM | 1.0 ± 0.6 (n = 4) | 2.7 ± 1.6 (n = 4) | 216.2 ± 197.5 (n = 3) | 14.8 ± 10.7 (n = 4) | 18.7 ± 8.9 (n = 3) |

**Table 1 Average $EC_{50}$ values from *in vitro* gene expression assays**

- Drug potency ranking in Huh-7 assay:
  T3 ≈ GC-1 > VK2809A >> MGL-3196 > VK2809
- Potencies are conserved in PHH for T3, GC-1, and MGL-3196
- VK2809 has significantly increased potency in PHH cells
  - PHH have CYP3A4 activity to cleave VK2809 (prodrug) into its parent form VK2809A, while HCC cell lines have reduced CYP450 expression[ii]

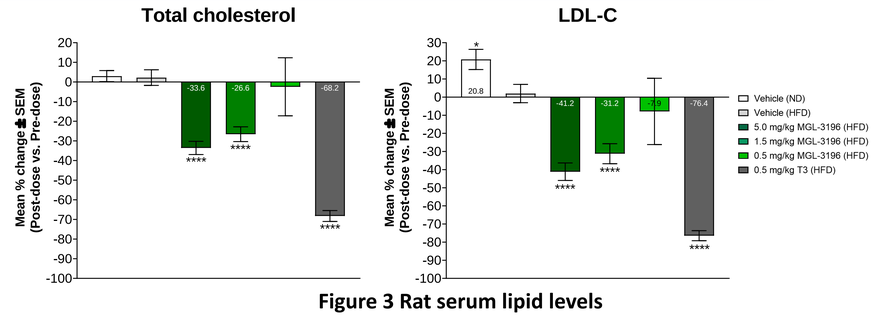


**Figure 3 Rat serum lipid levels**

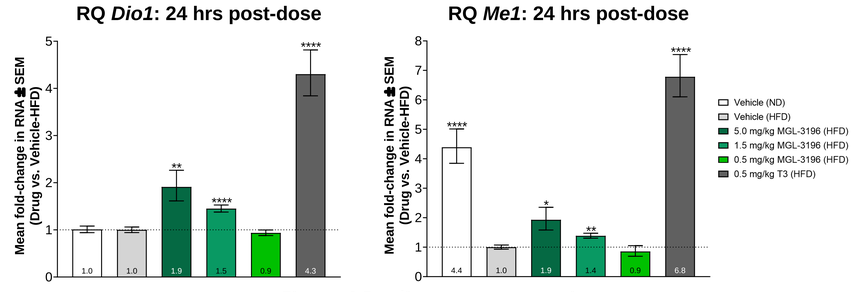


**Figure 4 Rat liver gene expression**

- 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
i. Sinha et al., Nat Rev Endocrinol. 2018 May; 14(5): 259–269. DOI: 10.1038/nrendo.2018.10
ii. Rodríguez-Antona et al., Xenobiotica. 2002 Jun;32(6):505-20. DOI: 10.1080/00498250210128675

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