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MASLD: Experimental and pathophysiology

ALG-055009, a potent and selective THRβ agonist for the treatment of MASH, induces pro-metabolic and anti-fibrotic gene expression in the liver of DIO mice

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ALIGOS THERAPEUTICS

BACKGROUND AND AIMS

Metabolic dysfunction-associated steatotic liver disease (MASLD) encompasses a heterogenous series of disorders ranging from fatty liver to more severe metabolic dysfunction-associated steatohepatitis (MASH). Thyroid hormone receptor beta (THRβ) is a clinically validated target for the treatment of MASH, with THRβ agonists able to selectively reduce fat deposits in the liver and potentially prevent the downstream consequences of MASLD (inflammation, fibrosis, cirrhosis, etc.). ALG-055009 (Fig. 1) is a THRβ agonist that has demonstrated significant reductions in liver fat (placebo-adjusted median relative reductions up to 46.2%) and atherogenic lipids in patients with presumed MASH and stage 1-3 liver fibrosis. Here, we present the effects of ALG-055009 in a diet-induced obese (DIO) mouse model and human liver cells.

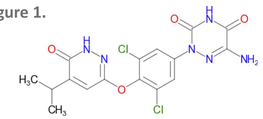
Phase 2a HERALD study highlights[1]

(see 2025 EASL presentation SAT-451 & posters SAT -430, -450, -451)

– Primary endpoint achieved with robust reductions in liver fat content at Week 12
- 70% of patients achieved >30% at 0.7 mg dose

– Significant reductions in atherogenic lipids (e.g. LDL-C, lipoprotein (a) & apolipoprotein b)
– Dose-dependent increases in SHBG (marker of THR-β activation in liver)
– Well-tolerated, with rates of GI-related AEs similar to placebo

Figure 1.



ALG-055009

METHODS

High Fat Diet-Induced Obese Mouse Study: C57BL/6J mice were fed with a high fat diet (HFD; D12492) for 14 weeks, followed by drug treatment for 4 weeks. ALG-055009 treatment groups included two oral QD dose levels of 0.5 and 1.5 mg/kg, and four oral BID dose levels ranging from 0.075 to 0.35 mg/kg/dose. Pharmacodynamic endpoints included total and low-density lipoprotein (LDL) cholesterol. Liver gene expression was monitored by RT-qPCR. Previously reported reductions in serum lipid levels are shown below (Table 1).

Table 1. High Fat Diet-Induced Obese Mouse Study

Group	Diet	Treatment	Dose (mg/kg, PO)	Dose Frequency	TC (mean % change)	LDL-C (mean % change)
1	ND	Vehicle	-	BID	-28.2****	-34.4**
2	HFD				0.0	0.0
3	HFD	ALG-055009	0.5	QD	-25.1****	-37.5***
4			1.5		-37.8****	-40.6***
5			0.075	BID	-9.6	-9.4
6			0.15		-17.2**	-34.4**
7			0.25		-34.4****	-59.4****
8			0.35		-44.3****	-56.3****

ND =normal chow diet (D12450J); HFD =high fat diet (D12492); PO =oral dosing; BID =twice daily; QD =once daily; n =6 animals per group; TC =total cholesterol; LDL-C =low-density lipoprotein cholesterol; statistical analysis: ordinary one-way ANOVA with Dunnet's multiple comparisons test (compared to HFD-Vehicle group at 28 days post-dose); ** =p-value <0.01; *** =p-value <0.001; **** =p-value <0.0001

In Vitro Gene Expression Assays: Huh-7 cells were cultured in media supplemented with 10% charcoal-stripped FBS and treated with vehicle or increasing concentrations of ALG-055009 or MGL-3196 for 24 hours. RNA was extracted and the resulting cDNA was used in RT-qPCR. Primary human hepatocytes (PHH) were plated, serum-starved for 24 hours, and then treated with vehicle, ALG-055009, or MGL-3196 for 24 hours at the indicated doses. RNA was extracted and was either used for cDNA library preparation and subsequent RNA-Seq or cDNA was prepared for use in downstream RT-qPCR analysis.

REFERENCES

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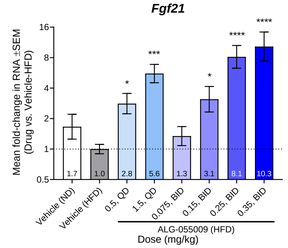
2) doi: 10.1016/j.metabol.2019.153994
3) doi: 10.1186/s11658-024-00675-6
4) doi: 10.1038/s41598-017-11212-1
5) doi: 10.1530/ETJ-22-0211
6) doi: 10.1152/ajpendo.90736.2008
7) doi: 10.1016/j.atherosclerosis.2022.04.006
8) doi: 10.1016/j.jcmgh.2019.10.010
9) doi: 10.1194/jlr.M700378-JLR200
10) doi: 10.1016/j.jhep.2006.02.011
11) doi: 10.3892/ijmm.2020.4479
12) doi: 10.1016/j.livres.2022.09.003
13) doi: 10.1016/j.jcmgh.2022.03.011
14) doi: 10.3390/ijms21062061
15) doi: 10.1038/srep45049

ALG-055009 Robustly Upregulates Liver *Fgf21* Expression in DIO Mice

Figure 2.



Values reported within bars indicate fold-change relative to HFD-Vehicle group at 28 days post-dose; statistical analysis: ordinary one-way ANOVA with Dunnet's multiple comparisons test (compared to HFD-Vehicle group); * =p-value <0.05; *** =p-value <0.001; **** =p-value <0.0001

***FGF21* (fibroblast growth factor 21): liver glucose and lipid metabolism[2]**

ALG-055009 Significantly Downregulates Pro-Fibrotic Liver Genes in DIO Mice

Figure 3a.

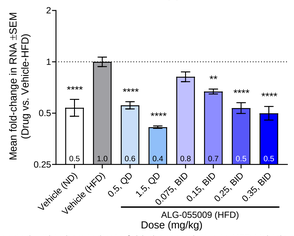
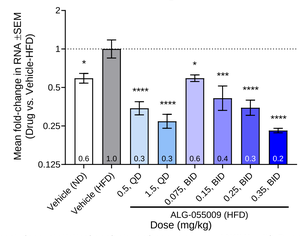


Figure 3b.



Values reported within bars indicate fold-change relative to HFD-Vehicle group at 28 days post-dose; statistical analysis: ordinary one-way ANOVA with Dunnet's multiple comparisons test (compared to HFD-Vehicle group); * =p-value <0.05; ** =p-value <0.01; *** =p-value <0.001; **** =p-value <0.0001

***Enpp2* (autotaxin): stellate cell activation and liver fibrosis[3]**

***Lgals1* (galectin-1): stellate cell activation and liver fibrosis[4]**

ALG-055009 May Increase Local Availability and Activity of Thyroid Hormone in the Liver of DIO Mice via Changes in Gene Expression

Figure 4a.

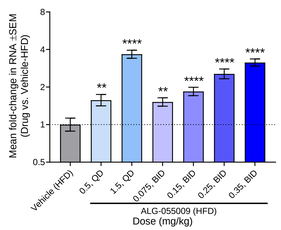
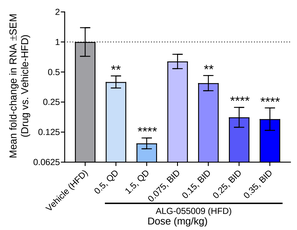


Figure 4b.



Values reported within bars indicate fold-change relative to HFD-Vehicle group at 28 days post-dose; statistical analysis: ordinary one-way ANOVA with Dunnet's multiple comparisons test (compared to HFD-Vehicle group); ** =p-value <0.01; **** =p-value <0.0001

***Dio1* (deiodinase, iodothyronine, type I): thyroid hormone activation and lipid metabolism[5]**

***Dio3* (deiodinase, iodothyronine type III): thyroid hormone inactivation and lipid metabolism[5]**

ALG-055009 is a Potent Regulator of Genes Involved in Lipid Transport

Figure 5a.

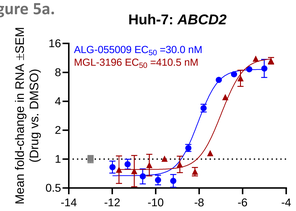
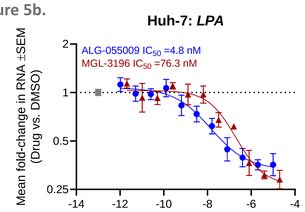


Figure 5b.



Values reported indicate fold-change relative to Vehicle group (■) at 24 hours post-dose; statistical analysis: nonlinear regression fit with variable slope

***ABCD2* (ATP binding cassette subfamily D member 2): fatty acid transport[6]** ***LPA* (lipoprotein (a)): cholesterol transport[7]**

ALG-055009 Alters Lipid and Collagen Metabolism in Primary Human Hepatocytes

Figure 6a.

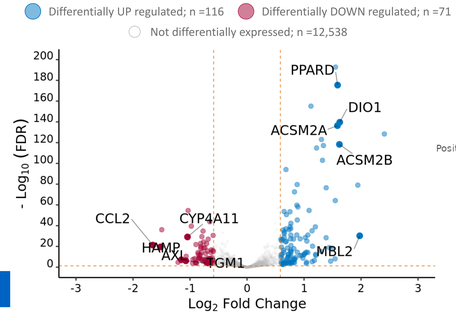
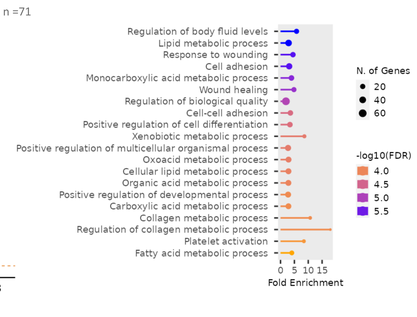


Figure 6b.



PHH =primary human hepatocytes; FDR =false discovery rate; differentially expressed defined as FDR <0.05 and $|\log_2\text{fold-change}| \geq 0.585$; GO =gene ontology

ALG-055009 Significantly Modulates Genes Relevant to MAFLD/MASH

Figure 7a.

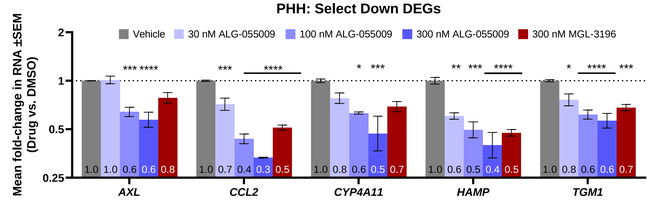
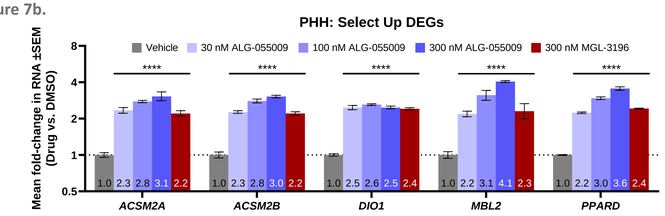


Figure 7b.



Values reported within bars indicate fold-change relative to Vehicle group at 24 hours post-dose; statistical analysis: ordinary one-way ANOVA with Dunnet's multiple comparisons test (compared to Vehicle group); * =p-value <0.05; ** =p-value <0.01; *** =p-value <0.001; **** =p-value <0.0001

***AXL* (AXL receptor tyrosine kinase): stellate cell activation & liver fibrosis[8]**

***ACSM2A/2B* (Acyl-CoA synthetase medium chain family member 2A/2B): activation of fatty acids[9]**

***CCL2* (C-C motif chemokine ligand 2): liver inflammation[10]**

***CYP4A11* (cytochrome P450 4A11): source of ROS in liver[11]**

***HAMP* (hepcidin antimicrobial peptide): liver iron metabolism[12]**

***MBL2* (mannose binding lectin 2): limit liver fibrosis progression[13]**

***PPARD* (peroxisome proliferator activated receptor δ): liver lipid metabolism and inflammation[14]**

***TGM1* (transglutaminase 1): liver fibrosis[15]**

CONCLUSIONS

Preclinical and clinical data demonstrate that ALG-055009 is positioned as a potential best-in-class THRβ agonist for the treatment of MASH. Here, we offer new evidence for the compound's mechanism of action. In DIO mice, ALG-055009 treatment increased liver *Fgf21* expression, suggesting improved lipid metabolism and insulin sensitivity. It also decreased *Dio3* and upregulated *Dio1* in the liver, potentially enhancing thyroid hormone activity, reducing hepatosteatosis, and curbing MASH progression. Additionally, ALG-055009 lowered the expression of pro-fibrotic markers, *Enpp2* and *Lgals1*. In Huh-7 cells, it is a potent regulator of genes involved in lipid transport. Genome-wide analysis in PHH via RNA-Seq revealed that ALG-055009 alters the expression of genes involved in lipid and collagen metabolic pathways, with the greatest and most significant effects on MASLD/MASH-related genes as confirmed by RT-qPCR. In both cell models, ALG-055009 was approximately 5X to 15X more potent than MGL-3196, reflecting its superior potency reported in preclinical rodent models and in patients in the clinic.

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