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Publication # 1039

Two Pre-clinical Short Interfering RNA Molecules Targeting Human HSD17beta13 for the Treatment of Metabolic Dysfunction-Associated Steatohepatitis

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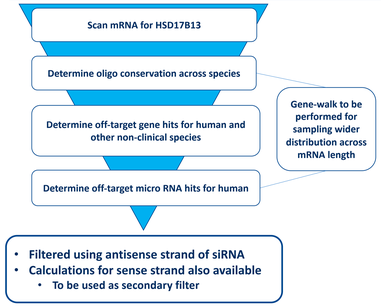
ALIGOS THERAPEUTICS

BACKGROUND

The liver-enriched, hepatocyte-specific, lipid droplet-associated protein 17-beta hydroxysteroid dehydrogenase isoform 13 (HSD17beta13 or HSD) is a strong genetic risk factor for the development and progression of metabolic dysfunction-associated steatotic liver disease (MASLD) into the more severe metabolic dysfunction-associated steatohepatitis (MASH). A loss-of-function splice variant rs72613567:TA is associated with decreased inflammation and liver injury in patients with fatty liver[1] and silencing of the gene via RNA interference resulted in decreased serum alanine aminotransferase levels in MASH patients[2]. The aim of our studies was to characterize short interfering RNA (siRNA) molecules that effectively silence the expression of HSD and could be used as potential therapies for MASLD/MASH.

METHODS



An on-/off-target luciferase reporter assay in Cos-7 cells and RT-qPCR assay in primary human hepatocytes (PHH) were used to screen proprietary human HSD-targeting siRNAs for effective silencing of target gene expression. Select compounds were then tested in PK/PD studies using human AAV-HSD mice, human knock-in (KI)-HSD mice, or non-human primates (NHP). Endpoints included target quantification via RT-qPCR and Western blot. RNA-Seq analysis of treated PHH was conducted to monitor siRNA selectivity and repeat-dose toxicity studies were conducted in rats to assess safety.

Inhibition of Human *HSD17B13* RNA *in vitro*

Figure 1a.

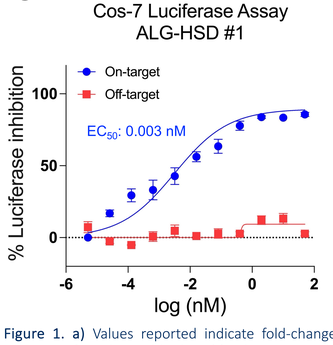


Figure 1b.

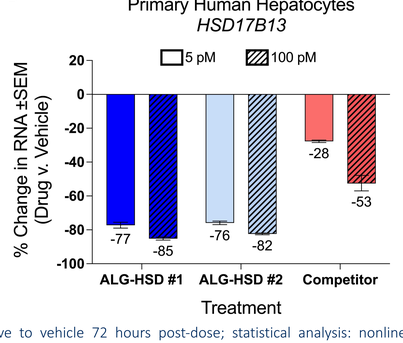


Figure 1. **a)** Values reported indicate fold-change relative to vehicle 72 hours post-dose; statistical analysis: nonlinear regression fit with variable slope **b)** Values reported below bars indicate % change relative to vehicle 48 hours post-dose

REFERENCES

1. Abul-Husn et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med. 2018 Mar 22;378(12):1096-1106. https://www.nejm.org/doi/10.1056/NEJMoa1712191
2. Mak et al. A phase I/II study of ARO-HSD, an RNA interference therapeutic, for the treatment of non-alcoholic steatohepatitis. J Hepatol . 2023 Apr;78(4):684-692. https://www.sciencedirect.com/science/article/abs/pii/S0168827822033207

Minimal Off-Target Activity in Primary Human Hepatocytes

Figure 2.

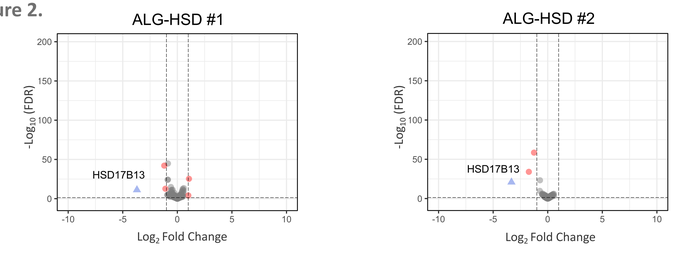


Figure 2. FDR =false discovery rate; differentially expressed defined as FDR <0.05 and |log_2 fold-change| >1

Inhibition of Human *HSD17B13* in AAV Mouse Model

Figure 3a.

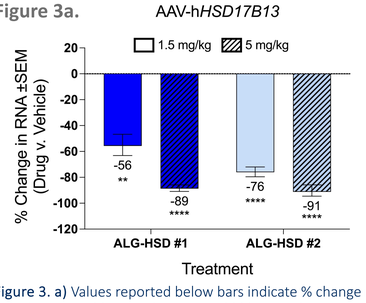


Figure 3b.

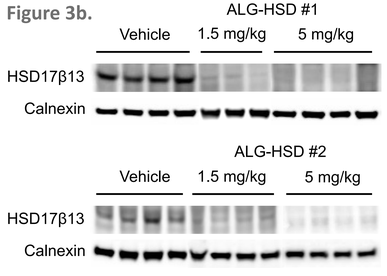


Figure 3. **a)** Values reported below bars indicate % change relative to vehicle 14 days post-dose; one-way ANOVA compared to Vehicle group; ** = p-value <0.01, **** = p-value <0.0001 **b)** Blots are probed with anti-HSD17β13; Calnexin was used as loading control

Inhibition of Human *HSD17B13* in Knock-In Mouse Model

Figure 4a.

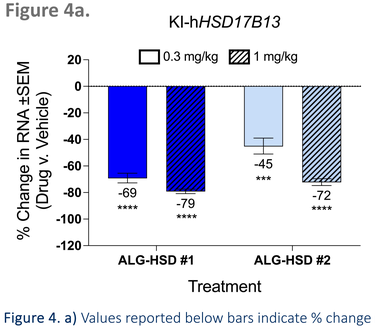


Figure 4b.

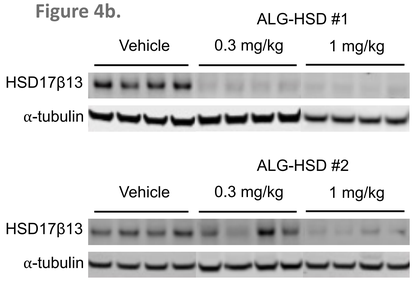


Figure 4. **a)** Values reported below bars indicate % change relative to vehicle 28 days post-dose; one-way ANOVA compared to Vehicle group; *** = p-value <0.001, **** = p-value <0.0001 **b)** Blots are probed with anti-HSD17β13; α-tubulin was used as loading control

Inhibition of *HSD17B13* in Non-Human Primates

Figure 5a.

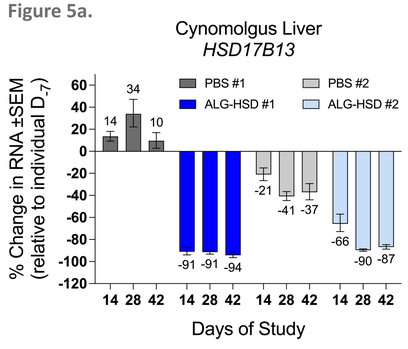


Figure 5b.

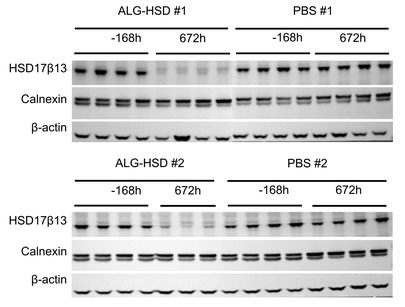


Figure 5. **a)** Values reported below bars indicate % change 14, 28, or 42 days post-dose relative to each individual animal's baseline (pre-dose) expression level **b)** Blots are probed with anti-HSD17β13; Calnexin and β-actin are used as loading control

Safety Profiles of Lead siRNAs in Rats

Figure 6.

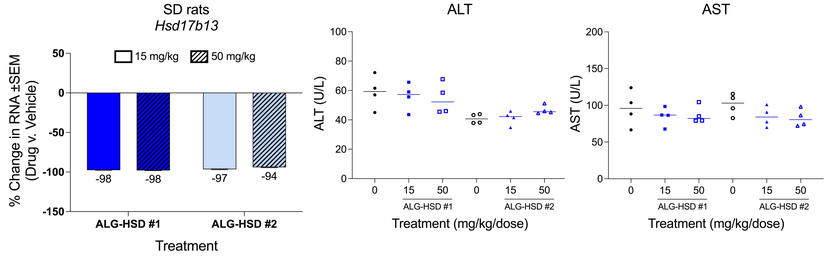


Figure 6. Values reported within bars indicate % change in RNA relative to vehicle group at 17 days post-dose. Individual values with median line are reported for each liver enzyme at 17 days post-dose; ALT = alanine aminotransferase; AST = aspartate aminotransferase

CONCLUSION

We discovered several potent siRNA molecules that selectively and effectively silence human *HSD17beta13* gene expression in vitro and in vivo. We implemented novel seed destabilization modifications to increase potency without compromising selectivity. The data generated in these studies allowed for the progression of select liver targeted GalNAc-conjugated siRNAs, ALG-HSD #1 and ALG-HSD #2, into later-stage preclinical studies.

Financial disclosures: Authors are or were employees of Aligos Therapeutics, Inc., or Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA, as indicated by the listed affiliations.