Two Pre-clinical Short Interfering RNA (siRNA) Molecules Targeting Human HSD17β13 for the Treatment of Metabolic Dysfunction-Associated Steatohepatitis

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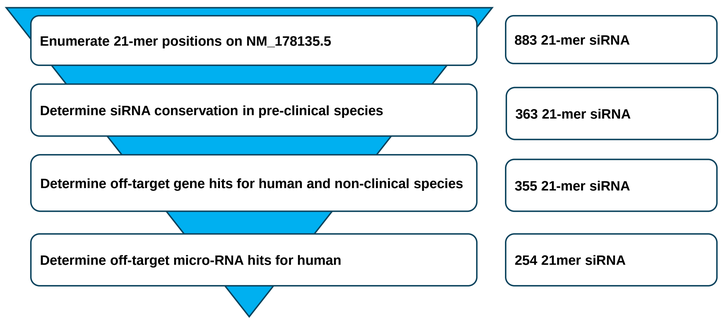
Introduction

- Metabolic Dysfunction Associated Steatotic Liver Disease (MASLD) is believed to affect approximately 250 million people (~30% of the world population)[1]
- Metabolic Dysfunction Associated Steatohepatitis (MASH) is a severe form of MASLD that is a leading cause for liver transplantation[1]
- First MASH treatment approved in 2024 (small molecule)[2]
- Previously, standard of care for MASH depended on lifestyle changes (diet, exercise, symptom treatment)
- Genome-Wide Association Study (GWAS) found loss-of-function splice variants in *HSD17β13* were strongly correlated with lower risk of liver diseases and inflammation (ALT/AST)[3]
- *HSD17β13* mRNA reduction correlates positively with improvement in nonalcoholic fatty liver disease activity score (NAS)[3]
- Multiple *HSD17β13*-targeting oligonucleotide therapeutics have undergone clinical evaluation (Phase 1-2)[4]

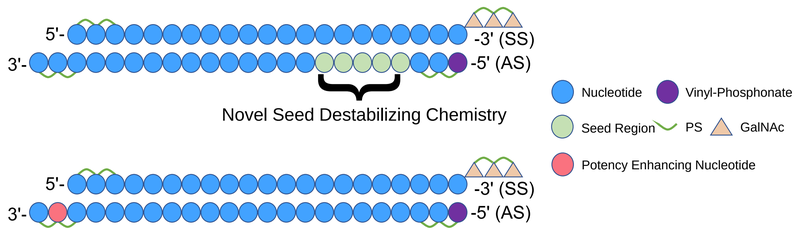
Objectives

- Identify new target regions and chemistries with improved *in vitro* and *in vivo* potency and maintain clean safety profiles
- Generate data to support progression of select ALG siRNAs into late-stage preclinical studies

In Silico Screening Operation and Hit Optimization Utilized Different Chemical Strategies

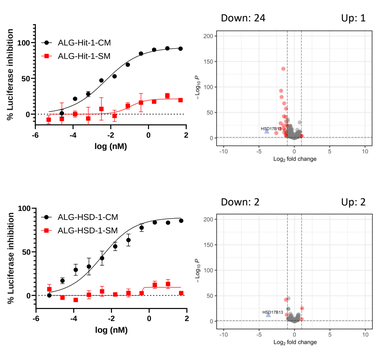


Hit:	Target Region	Liability	Chemistry Optimization	Final Leads
ALG-Hit-1	1	Selectivity	Novel Chemistry Position 7	**ALG-HSD-1**
ALG-Hit-2	2	Potency	Stabilizing 2'-OMe/F Pattern	**ALG-HSD-2**



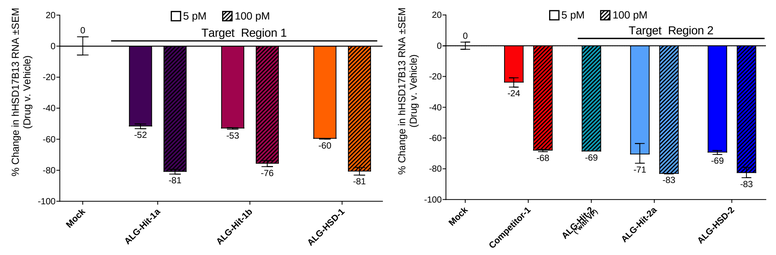
Hit siRNAs Displayed Varying Levels of Selectivity *In Vitro*

Hit:	Target Region	# of Differentially Expressed Genes (DEG)
ALG-Hit-1	1	25
ALG-Hit-1a	1	1
ALG-Hit-1b	1	2
ALG-HSD-1	1	4
ALG-Hit-2	2	2
ALG-Hit-2a	2	2
ALG-HSD-2	2	4
Competitor 1		4



- Luciferase constructs used to differentiate efficacy and selectivity:
  - SM construct has a region complementary to the siRNA AS only in the seed region
  - CM construct has a region complementary to the whole siRNA AS
  - Relative Luciferase activity indicates promiscuity of siRNA with limited sequence recognition
- Fold change for DEG (red circles) was > 2; approximately 16,000 genes analyzed
  - RNA-Seq found ALG-Hit-1 displayed promiscuous silencing
- Competitor-1 synthesized based on public information about ARO-HSD[4]

ALG Hits Potently Knockdown h*HSD17β13* in Primary Human Hepatocytes



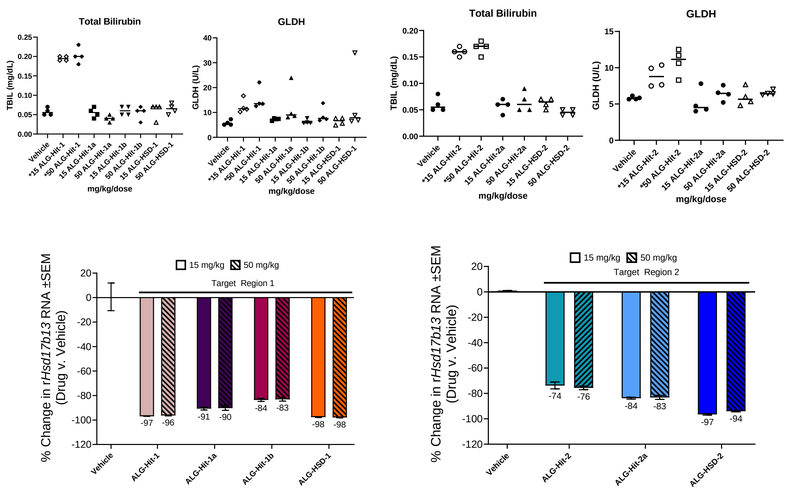
- RT-qPCR in primary human hepatocytes (PHH) used to screen siRNA (5 pM or 100 pM dose)
- Target Region 1 hits all near-equipotent at both doses; Head-to-head comparison found **ALG-HSD-1** outperformed Competitor-1 at both 5 and 100 pM in a separate study
- Target Region 2 ALG-Hit-2a and **ALG-HSD-2** achieved potency parity with Competitor-1 at a lower dose

ALG Hits Surpass Competitor in AAV Mouse Model

Dose	RNA Knockdown (%) h*HSD17β13*							
	Study of Target Region 1					Study of Target Region 2		
	Compe-titor-1	ALG-Hit-1	ALG-Hit-1a	ALG-Hit-1b	**ALG-HSD-1**	Compe-titor-1	ALG-Hit-2a	**ALG-HSD-2**
1.5 mg/kg	-33%	-76%	-50%	-29%	-56%	-53%	-63%	-76%
5 mg/kg	-57%	-86%	-79%	-79%	-89%	-85%	-87%	-91%

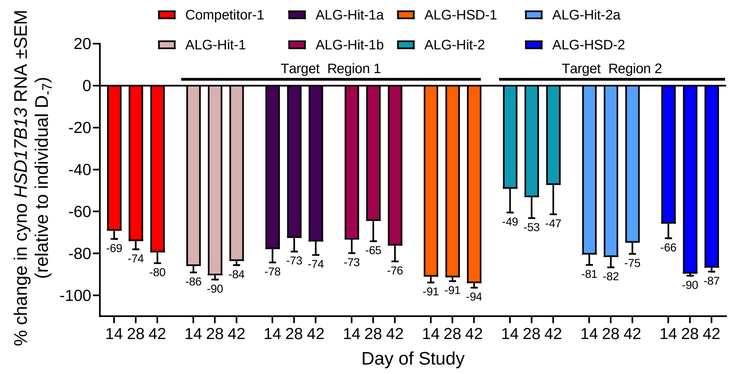
- AAV-HSD Mouse Model: 4 animals per dose range (1.5 or 5 mg/kg and a no-drug vehicle)
- Day 0 infection, Day 7 SC injection of siRNA, Day 21 collect liver and plasma for analysis
- Differential h*HSD17β13* expression generated by RT-qPCR
  - Enhancements of 10+% in RNA KD over Competitor-1 observed
- Differential expression of hHSD17β13 protein generated by western blot
  - WB data correlates well with RNA KD

Optimized Chemistry Contributed to Reduced Safety Liabilities and Robustly Enhanced Potency in Rats

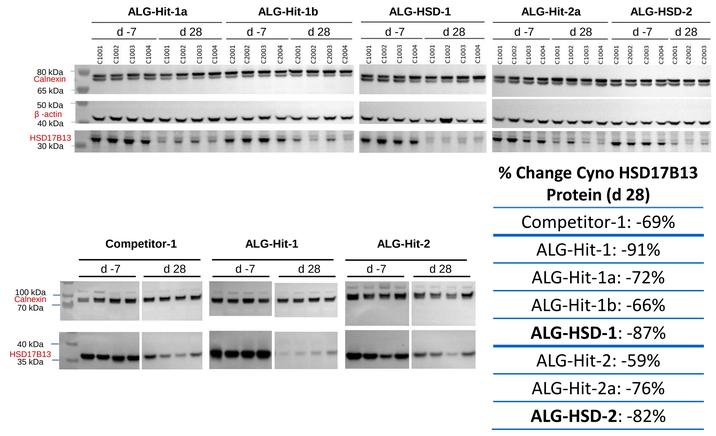


- Wistar-Han Rat Model: 4 animals per dose range (15 or 50 mg/kg and a no-drug vehicle)
- Day 1, 8, and 15 dose; terminal tissue and serum were collected for analysis on day 16
- **ALG-HSD-1** and **ALG-HSD-2** showed excellent potency (note: 2 MM in rat *HSD17β13* gene)
- Minimal to mild changes in body weight, albumin/globulin ratio, serum lipid concentrations, and liver enzymes were observed

ALG-HSD-1 and ALG-HSD-2 Strongly Reduced HSD17B13 RNA and Protein in a Non-Human Primate Model and Showed Enhanced Stability



- Male Cyno Monkey Model: 4 animals per group dosed with a single siRNA at 3 mg/kg or no-drug vehicle
- Plasma sampled over 48 hours after dosing; liver biopsy samples collected on days 14 and 42; clinical pathology samples collected at days -7, 1, 2, 14, 28, and 42 days
- Target region 1 siRNA show strong RNA knockdown; ALG-Hit-1 and **ALG-HSD-1** are the most potent in the series
- Target region 2 siRNA show dramatic efficacy enhancement with chemistry modifications; ALG-Hit-2a and **ALG-HSD-2** superior to ALG-Hit-2 parent



% Change Cyno HSD17B13 Protein (d 28)
Competitor-1: -69%
ALG-Hit-1: -91%
ALG-Hit-1a: -72%
ALG-Hit-1b: -66%
ALG-HSD-1: -87%
ALG-Hit-2: -59%
ALG-Hit-2a: -76%
ALG-HSD-2: -82%

- Western blot (WB) of HSD17B13 on liver samples collected on day -7 and day 28
- HSD17B13 levels calculated relative to loading control calnexin (and β-actin)
- Protein knockdown date correlates well with observed RNA knockdown, corroborating the potency observed by qPCR

Observed Metabolism	Relative Abundance to Full-Length Peak Area				
	ALG-Hit-1a	ALG-Hit-1b	**ALG-HSD-1**	ALG-Hit-2a	**ALG-HSD-2**
(n-1 at 3') + Deamination	124%	190.4%	24.2%	-	-
(n-1 at 3')	132%	115.5%	94.8%	64.8%	1.6%
Deamination	69.8%	132.7%	17.3%	-	-

- No significant clinical pathology changes were observed
- Differences in metabolic profile attributed to unique chemistry of each siRNA
- Target region 1 siRNA more prone to deamination and nuclease cleavage
- Target region 2 siRNA protected against nucleases with novel chemical modifications

Conclusion

- We report here data that support the progression of **ALG-HSD-1** and **ALG-HSD-2** into late-stage preclinical studies
- **ALG-HSD-1** maintained the exquisite potency of parent **ALG-Hit-1** while significantly reducing safety and selectivity liabilities
- **ALG-HSD-2** maintained the selectivity and safety of parent ALG-Hit-2 while greatly enhancing potency and metabolic stability

References

1. AASLD.org
2. https://clinicaltrials.gov/study/NCT03900429
3. N. Engl. J. Med. 2018, 378, 1096-1106
4. J. Hepatol. 2023, 78, 684-692; https://clinicaltrials.gov/study/NCT05519475

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