Discovery and Optimization of the Pre-Clinical Efficacy of Human PNPLA3-Targeting Short Interfering RNA Molecules (siRNAs) for the Treatment of Metabolic Dysfunction-Associated Steatohepatitis

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Introduction

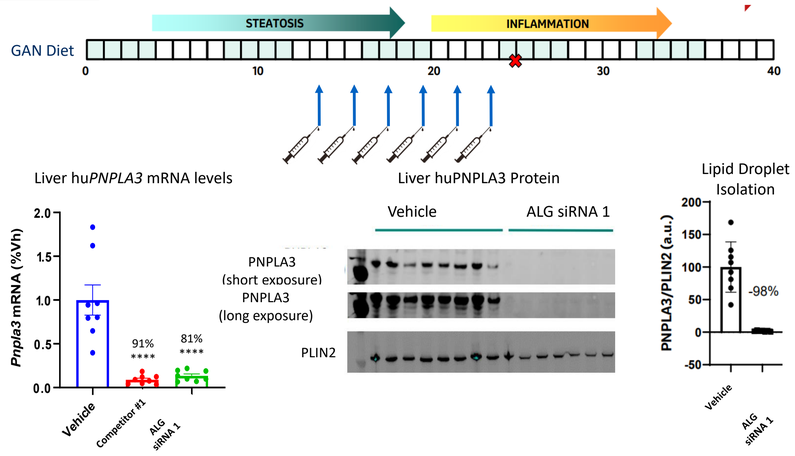
- Patatin-like phospholipase domain-containing protein 3 (PNPLA3), specifically the single nucleotide polymorphism rs738409[G] (I148M) variant, is a strong genetic risk factor for the development and progression of metabolic dysfunction-associated steatotic liver disease (MASLD)[1].
- Recently, resmetirom, a small molecule THRβ agonist, has been approved for the treatment of metabolic dysfunction-associated steatohepatitis (MASH). Additional treatment options, including siRNAs are of interest.
- Both ASO and siRNA approaches have been rapidly advancing in recent years to develop more effective and targeted alternatives for the treatment of MASH[2].
- The aim of our studies was to characterize short interfering RNA (siRNA) molecules that effectively silence the expression of PNPLA3 and could be used as potential therapies for MASLD/MASH.
- Aligos strategy: Target *PNPLA3* gene using siRNA with our proprietary designs.

Current Status of Aligos Proprietay siRNAs

sIPNPLA3	Target region	Goal for Improvement	Aligos Novel Chemistry	Status
ALG siRNA 1	#1 - Target bp	Improve potency	2'F/ 2'-OMe stabilization pattern	Ready for NHP DRF study
ALG siRNA 2	#2 - Target bp	Improve selectivity	Novel seed de-stablization chemistry	Late stage pre-clinical studies

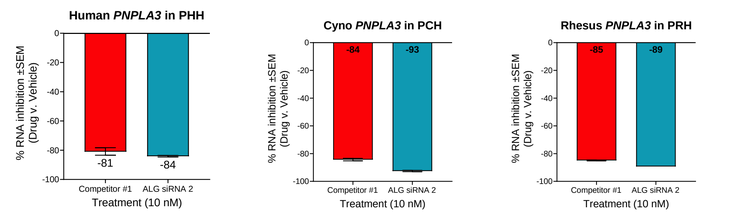
ALG siRNA 1: Inhibition of Human PNPLA3 in DIO Mice

Study Design:
- Human PNPLA3(I148M)-KI; male; GAN diet-fed
- Dosing regimen: 5.0 mg/kg, Q2W, S.C.
- Measurements: BW weekly; blood chemistry [week 4, week 8, week 12 (terminal)]
- **Endpoints:** PD (KD efficiency); Histology (MASH & Fibrosis)



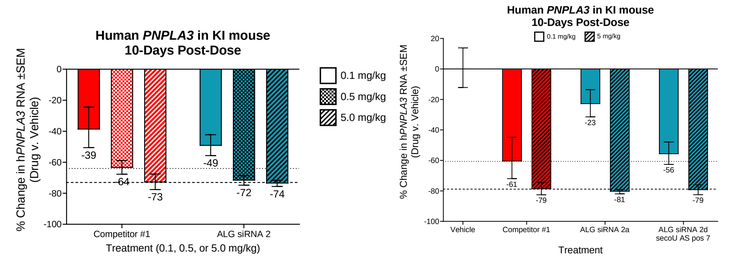
- Mice were fed GAN diet for 12 weeks and then dosed with siRNAs for 12 weeks at 5 mg/kg, Q2W, S.C.
- ALG siRNA 1 demonstrated robust RNA and protein knockdown of the human target in h*PNPLA3* KI mice.

ALG siRNA2: Conserved Activity Against Primate Targets



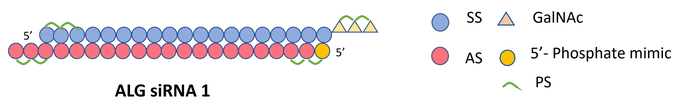
Primary cells (PHH; primary human hepatocytes, PCH; primary cyno hepatocytes, PRH; primary rhesus hepatocytes) were seeded in collagen coated multiwell plates. Twenty-four hours after seeding, cells were transfected with PBS (mock control)/1 or 10 nM siRNAs for 48 hr. The cells were harvested 48 hr after transfection for RNA extraction followed by RT-qPCR to test target expression.

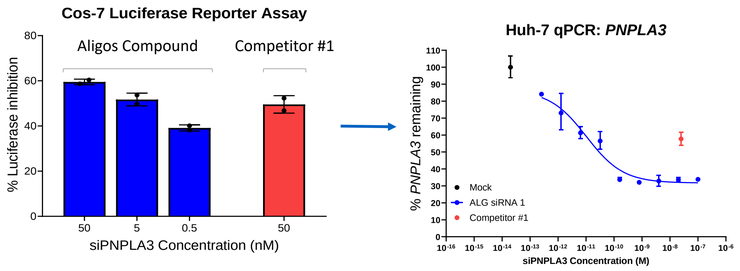
ALG siRNA 2: Robust Inhibition of Human PNPLA3 in KI Mice



- Seed destabilization with secoU @ position 7 on AS strand leads to a comparable potency as competitor control; unU @ position 6 has reduced potency at low dose
- Lead sequence @ position #2: potent in vitro activity on endogenous human target in PHH and favorable in vivo PK/PD properties (KI mouse)
- Lead sequence @ position #2: conserved in vitro activity on endogenous cyno and rhesus targets in primary monkey hepatocytes

ALG siRNA 1: Inhibition of Human PNPLA3 In Vitro

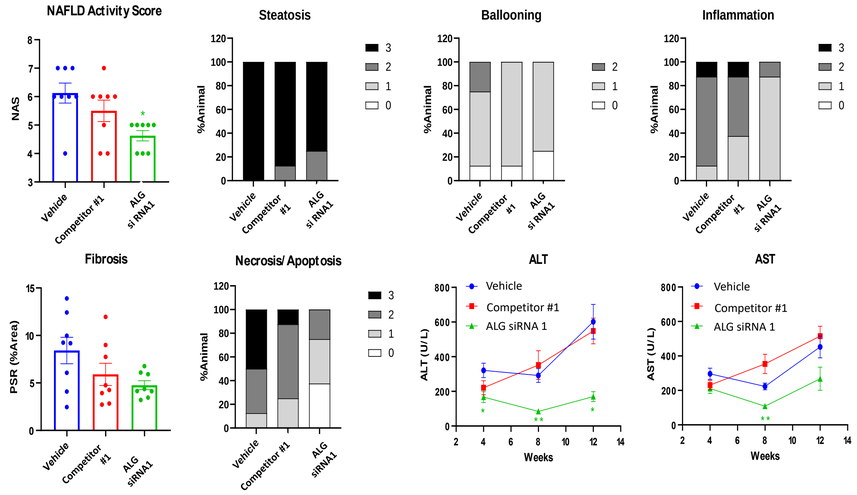




"Hit-to-Lead" selection using primary *in vitro* screens

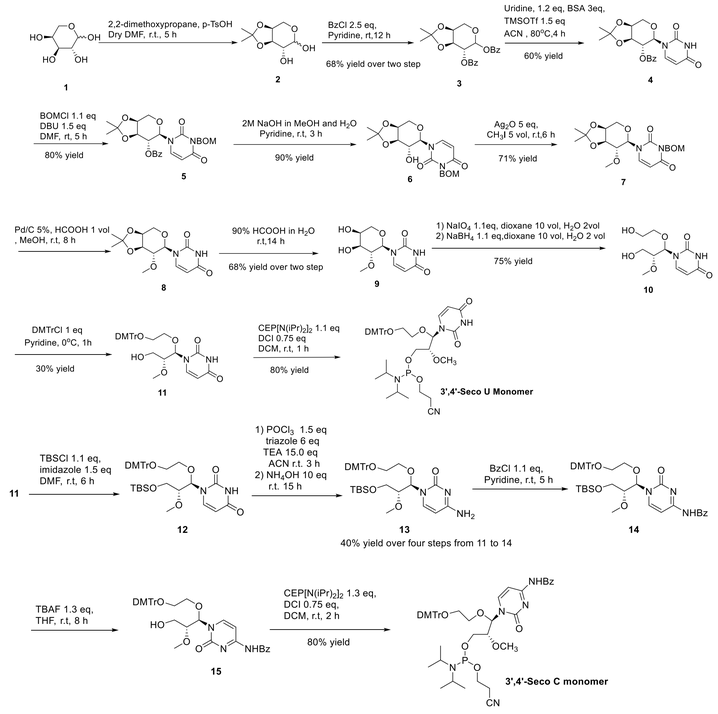
- ALG siRNAs show similar/better *in vitro* activity than competitor #1 .
- *PNPLA3* EC_{50} of ALG siRNA 1 = 0.008 nM, approximately 3-fold more potent than control #1.
- No significant cytotoxicity in Cos-7 or Huh-7 cells at the concentrations tested.

ALG siRNA 1: Improvements of NAS Endpoints in DIO Mice



- Using the DIO h*PNPLA3* KI mouse model, treatment with ALG siRNA 1 resulted in the greatest reductions/improvements in MASH histological endpoints (NAS score), fibrosis (PSR % area), and necrosis/apoptosis.
- In parallel, ALG siRNA 1 showed significant reductions in liver enzyme levels.

Synthesis of 3',4'-Seco Monomers

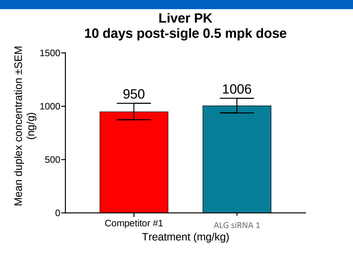


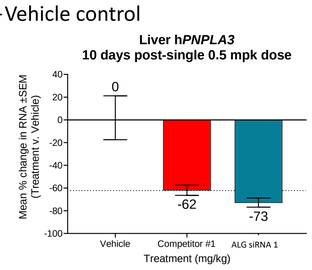
ALG siRNA 1: Inhibition of Human PNPLA3 in KI Mice

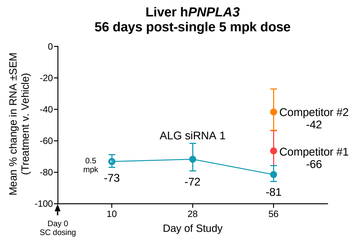
C57BL/6J h*PNPLA3 I148M* KI mice
(n=6 per group)

Day 0 (SC dose)
- 0.5 or 5.0 mg/kg ALG siRNA 1
- Vehicle control

Day 10 or Day 56
Termination

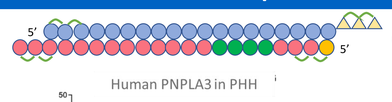




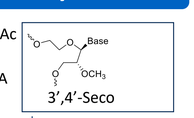


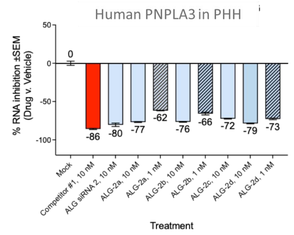
- A h*PNPLA3* I148M KI mouse model was used to assess the PK/PD and durability of ALG siRNA 1 target knockdown
- ALG siRNA 1 has favorable *in vivo* PK/PD properties comparable/better than competitor controls in the h*PNPLA3* KI mouse model
- ALG siRNA 1 retained activity 56 days post-dose after a single dose

ALG siRNA 2: Optimizations Improved In Vitro Selectivity

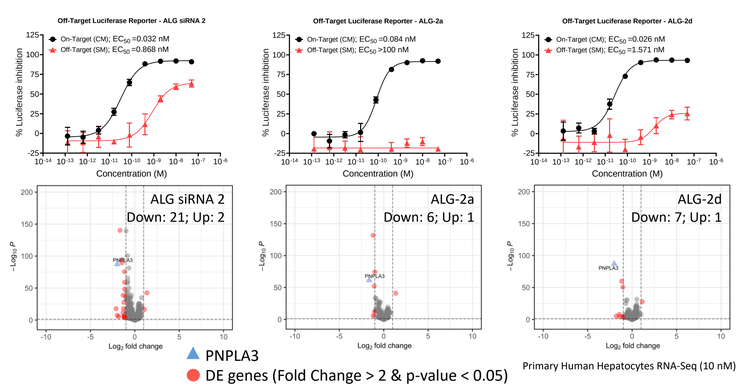


SS AS 5'- Phosphate mimic Seed chemistry GalNAc PS UNA un





ALG siRNA 2	AS Seed Chemistry	# of Differentially Expressed Genes
ALG siRNA 2	Parent, N/A	23
ALG-2a	un U pos 6	7
ALG-2b	un U pos 7	39
ALG-2c	3',4'-Seco U pos 6	38
ALG-2d	3',4'-Seco U pos 7	8



Conclusions

- We discovered several siRNA molecules that effectively silence human PNPLA3 gene expression in vitro and in vivo.
- Repeat dosing of ALG siRNA 1 in a DIO mouse model ultimately led to the improvement of MASH endpoints.
- Optimized siRNA ALG-2d demonstrated robust, dose-dependent knockdown of the human RNA target in PK/PD studies using human PNPLA3 KI mice.
- Novel Seco seed destabilization chemistry led to improved off target profile
- Further common genes, gene ontology, and pathway analysis ongoing
- No cytotoxicity was observed for all tested siRNA up to the highest tested concentration of 1000 pM.
- The data generated in these studies allowed for the progression of select ALG siRNAs into late-stage preclinical studies.

References

1. Liver International. 2023;43:975–988.
2. Journal of Hepatology, October 2023. vol. 79 j 1056–1064

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