In vitro and in vivo pharmacological characterization of human PNPLA3-targeting short interfering RNA molecules for the treatment of metabolic dysfunction-associated steatohepatitis

Abstract THU-198

Jieun Song[1], Jerome Deval[1], Lillian Adame[1], Ruchika Jaisinghani[1], Emily Tso[2], Daniel Lin[2], Aneerban Bhattacharya[1], Antitsa Stoycheva[1], Jacquelyn Sousa[1], Craig Parish[2], Saul Martinez Montero[1], Vivek Rajwanshi[1], Shane Daguison[1], Vikrant Gohil[1], Qingling Zhang[1], Toni Williamson[2], Sushmita Chanda[1], Saswata Talukdar[2], David B. Smith[1], Leonid Beigelman[1], Julian Symons[1], Yingjiang Zhou[2], Xuan Luong[1]*

[1]Aligos Therapeutics, Inc., South San Francisco, CA, United States; [2]Merck & Co., Inc., Rahway, NJ, United States, **Corresponding author: xluong@aligos.com*

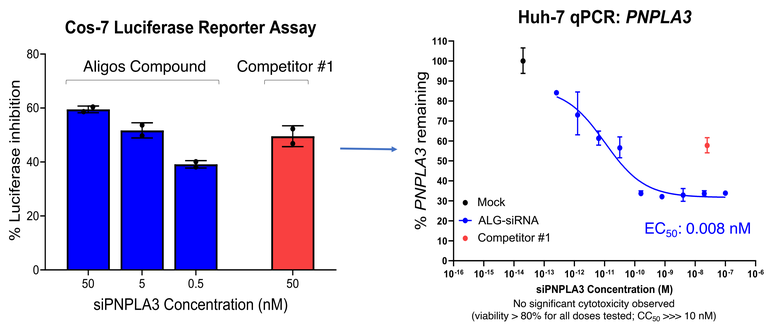
Background and Aims

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) into the more severe metabolic dysfunction-associated steatohepatitis (MASH). While resmetirom, a small molecule THRβ agonist, has recently been approved for the treatment of MASH, the field of oligonucleotide-based therapeutics has been rapidly advancing in recent years to develop more effective and targeted alternatives, with several drugs achieving market approval and many more in clinical trials for metabolic indications.[1] 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.

Methods

A luciferase reporter assay in COS-7 cells and RT-qPCR assay in Huh-7 cells were used to initially screen proprietary human *PNPLA3* (h*PNPLA3*)-targeting siRNAs *in vitro* for effective silencing of *PNPLA3* gene expression. Select compounds were then tested *in vivo* in PK/PD studies using h*PNPLA3* knock-in (KI) mice and a subset of molecules was then characterized in MASH efficacy studies using GAN diet-induced obese (DIO), h*PNPLA3* knock-in mice. Endpoints included target quantification via RT-qPCR and Western blot analysis, blood liver enzyme levels, and liver histology.

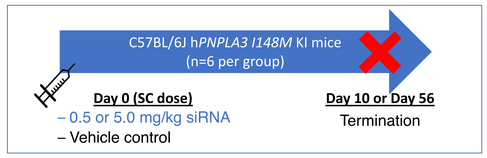
Inhibition of Human *PNPLA3* RNA *In Vitro*

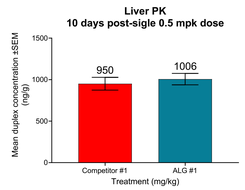


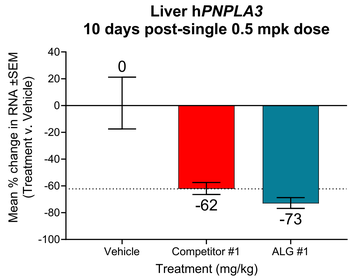
"Hit-to-Lead" selection using primary *in vitro* screens

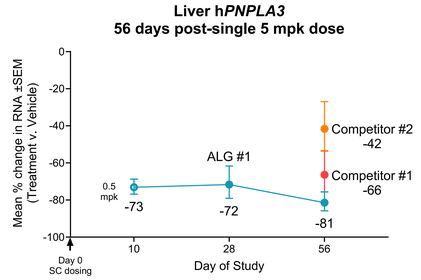
- **Luciferase reporter assay: ALG siRNAs show similar/better *in vitro* activity than competitor control**
- **Huh-7 RT-qPCR assay: *PNPLA3* EC_{50} of ALG siRNA = 0.008 nM**
  - **Approximately 3X more potent than competitor control #1**
- **No ALG siRNAs exhibited significant cytotoxicity in Cos-7 or Huh-7 cells at the concentrations tested**

Inhibition of Human *PNPLA3* RNA in PK/PD Mouse Model







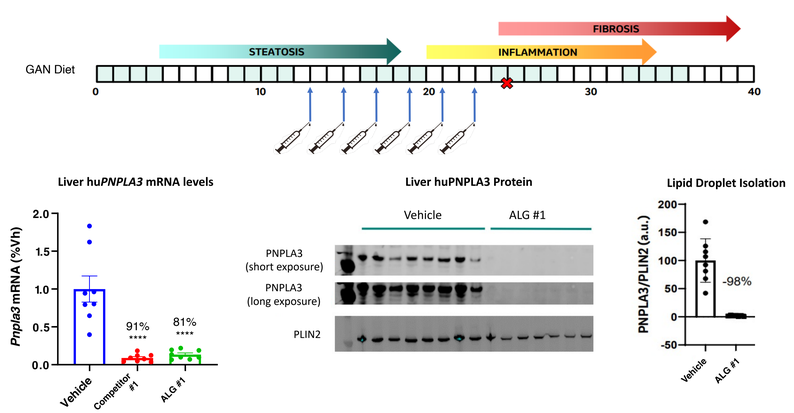


- **A h*PNPLA3* I148M KI mouse model was used to assess the PK/PD and durability of ALG siRNAs**
- **ALG #1 has favorable *in vivo* PK/PD properties comparable/better than competitor controls in the h*PNPLA3* KI mouse model**
- **ALG #1 retained activity 56 days post-dose after a single dose**

Inhibition of Human PNPLA3 RNA and Protein in the DIO Mouse Model

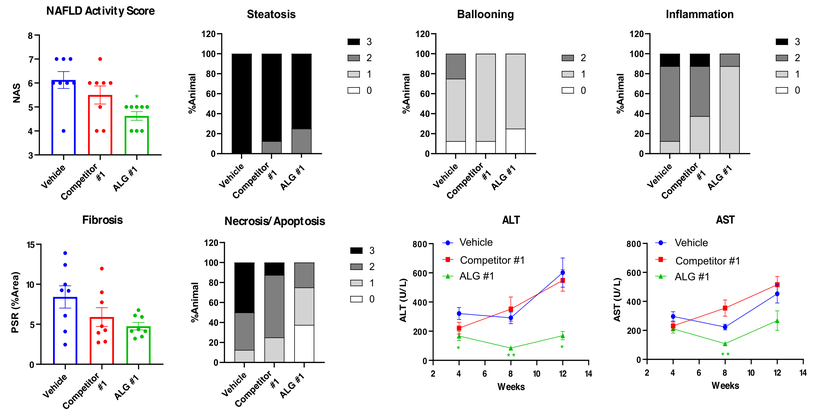
Study Design:

- Human PNPLA3(I148M)-KI; male; GAN diet-fed
- Dosing regimen: 5.0 mpk, 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 #1 demonstrated robust RNA and protein knockdown of the human target in h*PNPLA3* KI mice**

Improvements of NAS Endpoints in the DIO Mouse Model



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

Results and Conclusions

In the luciferase reporter assay, ALG siRNAs showed target inhibition greater than 50% at concentrations of 5 to 50 nM. In the RT-qPCR assay, ALG siRNAs inhibited the endogenous expression of *PNPLA3* with half maximal effective concentrations (EC_{50}) ranging between 1 and 60 pM. Select siRNAs demonstrated robust, dose-dependent knockdown of the human RNA target in PK/PD studies using human PNPLA3 KI mice. These effects were observed after a single dose at concentrations as low as 0.1 mg/kg (data not shown; 0.5 mg/kg data presented) and silencing of gene expression could be sustained for up to 56 days post-dose. Furthermore, repeat dosing of compounds in DIO mice led to robust target RNA and protein knockdown; one select compound was shown to also decrease in liver enzyme levels, improve MAFLD Activity Score (NAS) score, and reduce liver fibrosis and necrosis/apoptosis in this model.

In conclusion, we discovered several siRNA molecules that effectively silence human *PNPLA3* gene expression *in vitro* and *in vivo* and repeat dosing of a select compound in a DIO mouse model ultimately led to the improvement of MASH endpoints. The data generated in these studies allowed for the progression of select ALG siRNAs into late-stage preclinical studies.

References:

1. Goga, A., Stoffel, M. Therapeutic RNA-silencing oligonucleotides in metabolic diseases. Nat Rev Drug Discov 21, 417–439 (2022). https://doi.org/10.1038/s41573-022-00407-5

Financial disclosures: Authors are 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.