TOP-357

Second generation HBV siRNAs with novel chemistries demonstrate improved profiles compared with ALG-125755 and other clinical stage siRNAs

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ALIGOS THERAPEUTICS

Introduction

Hepatitis B virus (HBV) siRNAs have been shown to effectively reduce HBsAg in chronic hepatitis B (CHB) subjects. When combined with interferon or a TLR7 agonist, a significant number of patients demonstrate HBsAg loss. ALG-125755 is an HBV siRNA currently in Phase I. Single doses have been evaluated in healthy volunteers and virologically suppressed HBeAg negative CHB subjects. ALG-125755 was well tolerated with a favorable PK profile and viral kinetic data indicated evidence of HBsAg lowering at all dose levels evaluated.[1]

Aim

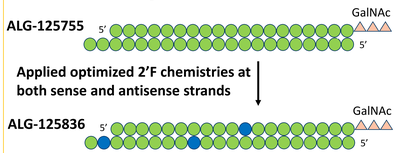
In this study, a multi-pronged approach was taken to further improve the potency, stability, hepatocyte-specific delivery and safety of ALG-125755 with the aim of developing a novel best-in-class HBV siRNA.

Method

In vitro inhibition of HBsAg release by siRNA was performed in the HepG2.2.15 cell line after transfection. Secreted HBsAg was quantified by ELISA. The stability of HBV siRNAs was profiled in mouse liver homogenates. Off target activity was evaluated by RNAseq in HepG2.2.15 cells. The binding of different GalNAc moieties to the asialoglycoprotein receptor (ASGR) was measured in human and mouse liver microsomes. In the AAV-HBV mouse model, HBV siRNAs conjugated with GalNAc were administered subcutaneously (SC) with serial blood collections for HBsAg and ALT assessment.

Results

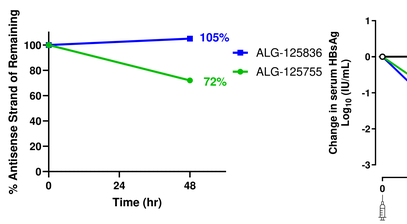
Optimized 2'F Chemistries Improved Stability and In Vitro/In Vivo Potency



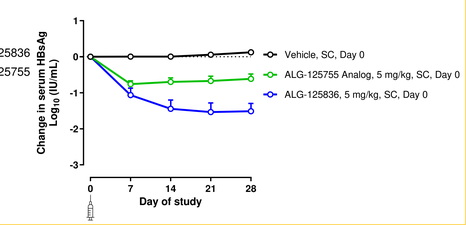
HepG2.2.15 HBsAg Release Inhibition Assay

siRNA ID	EC_{50} (pM)	CC_{50} (pM)
ALG-125755	12	>1000
ALG-125836	2.2	>1000

Mouse Liver Homogenate Stability Assay



AAV-HBV Mouse Model: Serum HBsAg Reduction

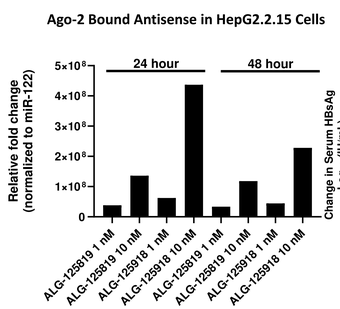


A Novel 5' End Cap Improved RISC Loading and In Vivo Potency

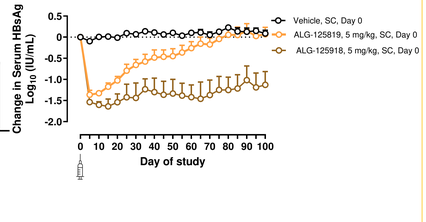
HepG2.2.15 HBsAg Release Inhibition Assay

siRNA ID	EC_{50} (pM)	CC_{50} (pM)
ALG-125819	8	>1000
ALG-125918	9	>1000

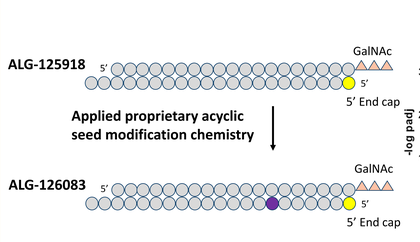
Ago-2 Bound Antisense in HepG2.2.15 Cells



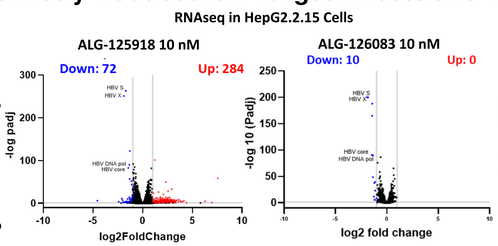
AAV-HBV Mouse Model: Serum HBsAg Reduction



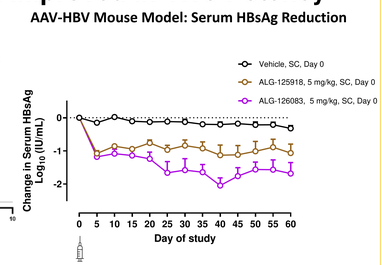
Novel Seed Modification Chemistry Reduced Off Target Effects and Improved In Vivo Potency



RNAseq in HepG2.2.15 Cells

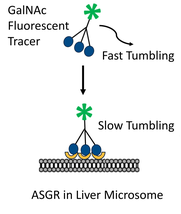


AAV-HBV Mouse Model: Serum HBsAg Reduction

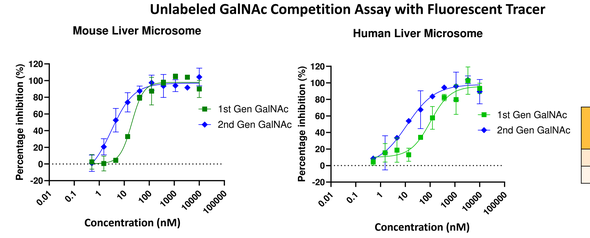


Novel GalNAc Moieties Improved Binding Capacity to the Human Asialoglycoprotein Receptor

Fluorescence Polarization Competition Assay



Unlabeled GalNAc Competition Assay with Fluorescent Tracer



IC_{50} for Replacement of Fluorescent Tracer

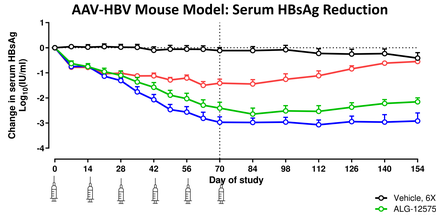
GalNAc	Mouse Liver Microsome IC_{50} (nM)	Human Liver Microsome IC_{50} (nM)
1st Gen	16	98
2nd Gen	5.9	14

ALG-125839 with Novel Chemistries: Current Lead Aligos 2nd Gen HBV siRNA

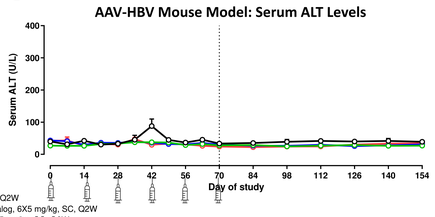
HepG2.2.15 HBsAg Release Inhibition Assay

siRNA ID	EC_{50} (pM)	CC_{50} (pM)
ALG-125755	12	>1000
ALG-125839	3.1	>1000

AAV-HBV Mouse Model: Serum HBsAg Reduction



AAV-HBV Mouse Model: Serum ALT Levels



Conclusions

1) We have built an siRNA platform incorporating novel chemistries that address different issues in the development of siRNAs.

2) ALG-125839 is a lead second-generation HBV siRNA that incorporated some of these novel chemistries. ALG-125839 demonstrated superior activities compared to the first-generation HBV siRNA ALG-125755 (Phase 1) and a competitor's Phase 2 HBV siRNA in an AAV-HBV mouse model. Further optimization is warranted.

References

1. JUCOV A. et. al. SAT 188 EASL, 2023

Disclosures

All authors are current or former Aligos Therapeutics, Inc., employees.