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

Jin Hong, Vivek K. Rajwanshi, Saul Martinez Montero, Min Luo, Kellan Passow, Cheng Liu, Hyunsoon Kang, Jacquelyn Sousa, Dana Cho, John Cortez, Shane Dagoussis, Kusum Gupta, Hua Tan, Vera Huang, Dawei Cai, Rostom Ahmed-Belkacem, Lawrence M. Blatt, David B. Smith, Leonid N. Beigelman, Julian A. Symons
Aligos Therapeutics, Inc., 1 Corporate Drive, South San Francisco, CA, USA

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 HepG2.2.15 cells. The binding of different GalNAc moieties to the asialoglycoprotein receptor (ASGR) was measured in Human Liver Microsome. In the AAV-HBV mouse model, HBV siRNA 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

Mouse Liver Homogeneity Stability assay

AAV-HBV Mouse Model: Serum HBsAg Reduction

ALG-125755

Applied optimized 2’F chemistry at both sense and antisense strands

ALG-125839

HepG2.2.15 HBsAg release inhibition assay

AAV-HBV Mouse Model: Serum HBsAg Reduction

ALG-125839

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

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

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

ALG-125918

Novel GalNAc Moieties Improved Binding Capacity to the Human Asialoglycoprotein Receptor

Fluorescence Polarization Competition assay

Unlabeled GalNAc Competition assay with fluorescent tracer

IC50 for replacement of fluorescent tracer

ALG-125918

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. JGID A et al. SAT 185 EASL, 2023

Disclosures

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