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Suppression of PD-L1 expression by a novel liver-targeted siRNA leads to potential restoration of immune responses against HBV

ALIGOS THERAPEUTICS

Dawei Cai, Saul Martinez Montero, Vera Huang, Megan E. Fitzgerald, Aneerban Bhattacharya, Lawrence M. Blatt, David B. Smith, Julian A. Symons, Leonid N. Beigelman, Jin Hong

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Aligos Therapeutics, Inc., 1 Corporate Drive, 2nd Floor, South San Francisco, CA 94080

Email: jhong@aligos.com

Background and Aim

Persistence of hepatitis B virus (HBV) infection in chronic hepatitis B (CHB) patients is associated with dampened HBV-specific T cell responses. Upregulation of programmed cell death protein 1 (PD-1) on T cells together with upregulation of programmed cell death-ligand 1 (PD-L1) on liver cells is considered as a primary cause. Therefore, suppression of PD-L1 expression may restore immune responses and trigger immune mediated clearance of HBV-infected hepatocytes, considered critical for CHB cure. We have developed a small interfering RNA (siRNA) platform technology using novel stabilization chemistries and applied this to both HBV and PD-L1 siRNA drug development. Comparing with PD-1 or PDL-1 antibody therapies, liver targeting PD-L1 siRNA could potentially reduce systemic toxicity.

Methods

The siRNAs targeting highly conserved regions between mouse and human PD-L1 were designed and the versions with N-acetylgalactosamine (GalNAc) conjugation were synthesized on MerMade synthesizers. The PD-L1 mRNA level of HBV infected PHH was evaluated in quad-culture Emulate Liver-Chip. In vitro siRNA PD-L1 knockdown was evaluated in the SNU-387 cell line by RT-qPCR. Animal PK/PD were studied in C57BL/6 mice with PD-L1 elevation induced Poly IC. GalNAc-siRNAs were dosed subcutaneously (SC). After treatment, mouse livers and kidneys were collected and subjected to evaluation of PD-L1 mRNA and protein by RT-qPCR and ELISA respectively. Liver siRNA level was measured with LCMS. In an ongoing AAV-HBV mouse study, GalNAc-siRNA was given via 8 weekly SC administrations and serum HBsAg/HBeAg and ALT were assessed weekly.

PD-L1 mRNA Elevated in HBV Infected PHH in 3-D Culture

- HBV specific T-Cell responses are impaired in CHB Patients
- PD-1 up-regulation was observed in HBV specific T cells
- PD-L1 up-regulation was observed in hepatocytes
- The combination of induction of PD-L1 expression by hepatocytes and elevated PD-1 level by CD8+ T cells is considered to play a critical role in T cell exhaustion during chronic HBV infection
- GalNAc-conjugated human PD-L1 siRNA could potentially be used to restore immune responses against HBV

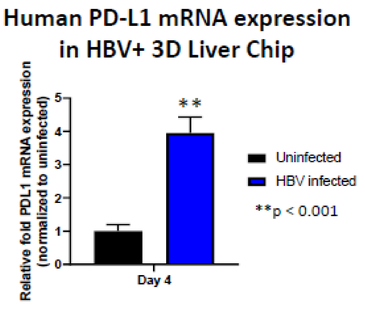


Figure 1. HBV induced higher PD-L1 in PHH

In Vitro Screening Identified siRNA "Hot Spot" in hPD-L1 Sequence

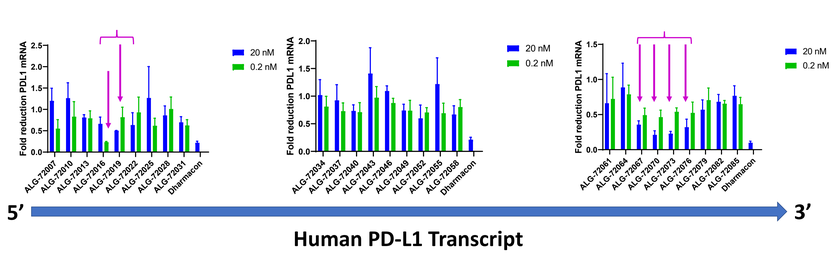


Figure 2. Target knockdown in SNU387 cells by fully stabilized hPD-L1 siRNA incorporating standard chemistries

Activity of 3 siRNA Located at Hot Spots

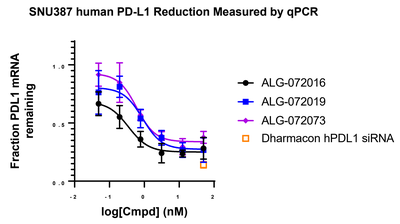
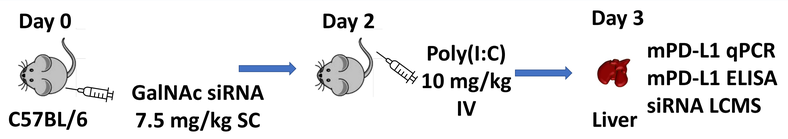


Figure 3. Dose response curves inhibiting hPD-L1 RNA in SNU387 cells

	EC_{50} (nM)	CC_{50} (nM)
ALG-072016	0.35	> 50
ALG-072019	0.89	> 50
ALG-072073	0.60	> 50

Table 1. EC_{50} and CC_{50} of PD-L1 siRNAs

PK/PD of GalNAc Conjugated PD-L1 siRNA



GalNAc siRNA	Unconjugated Parent	Liver mPD-L1 RNA Knockdown	Liver mPD-L1 Protein Knockdown	Liver siRNA Conc. (ng/g)
ALG-072143	ALG-072016	46%	50%	2,577
ALG-072144	ALG-072019	30%	20%	15,667
ALG-072148	ALG-072073	46%	5%	6,777

Table 2. PD-L1 siRNA PK/PD results

Initial AAV-HBV Mouse Study with Unoptimized PD-L1 siRNA

GalNAc siRNA	Dose	Max Serum HBsAg Reduction	Max HBeAg Reduction	ALT Elevation
ALG-072143	5X7.5 mg/kg QW	-0.5 log_{10} IU/ml	-0.14 log_{10} IU/ml	None
ALG-072144	5X7.5 mg/kg QW	-0.64 log_{10} IU/ml	-0.13 log_{10} IU/ml	None
ALG-072148	5X7.5 mg/kg QW	-0.9 log_{10} IU/ml	-0.13 log_{10} IU/ml	None

Table 3 Unoptimized PD-L1 siRNA efficacy study in AAV-HBV mouse model

ALG-072571: Aligos Chemistry Improved In Vitro Potency 10-fold

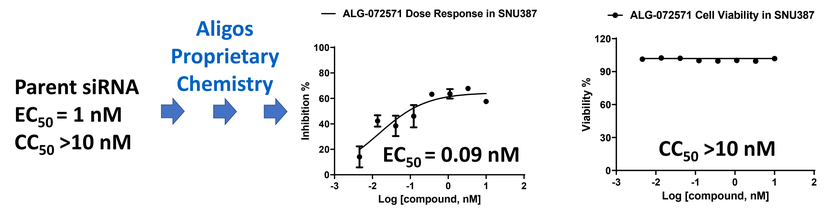


Figure 4. Activity of optimized hPD-L1 siRNA incorporating Aligos proprietary chemistry

ALG-072571: Aligos Chemistry Improved In Vivo Potency

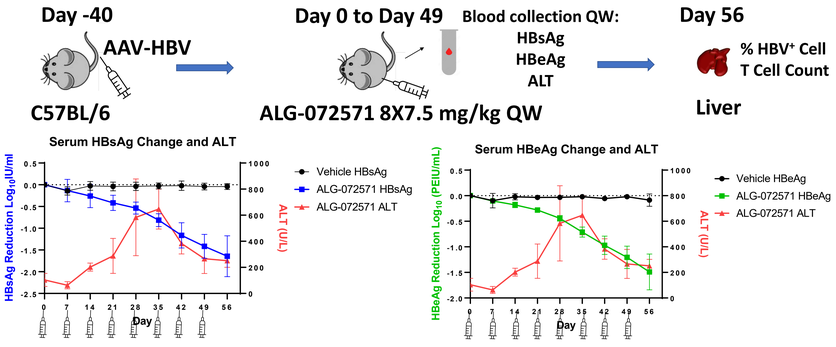


Figure 5. ALG-072571 significantly reduced HBsAg and HBeAg following ALT elevation

Conclusions

ALG-072571 is a human PD-L1 siRNA incorporating Aligos proprietary stabilization chemistries. The compound significantly reduced serum HBsAg and HBeAg in the AAV-HBV mouse model following ALT elevation. Further characterization of this siRNA is ongoing.

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