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

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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. 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.

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

Activity of 3 siRNA Located at Hot Spots

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

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

Table 1. EC50 and CC50 of PD-L1 siRNAs

Table 2. PD-L1 siRNA PK/PD results

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

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.

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

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

Figure 6. ALG-072571 significantly reduced HBsAg and HBeAg following ALT elevation

Technical aspects: The Aligos proprietary chemistries were synthesized on MerMade synthesizers. The compound significantly reduced serum HBsAg and HBeAg in the AAV-HBV mouse model following ALT elevation. Further characterization of this siRNA is ongoing.

Figure 1. HBV induced higher PD-L1 in PHH

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

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