A potent human PD-L1 siRNA leads to significant reduction of AAV-HBV infected hepatocytes via immune activation in human PD-1/PD-L1 double knock in mice

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EASL CONGRESS 2023
Vienna, Austria
21–24 June

FRI-239

ALIGOS THERAPEUTICS

BACKGROUND AND AIMS

T cell exhaustion is characteristic of chronic hepatitis B (CHB) and contributes to the persistence of hepatitis B virus (HBV) infection. PD-1/PD-L1 is the dominant co-inhibitory axis mediating T cell exhaustion in CHB patients. Monoclonal antibodies against PD-1 or PD-L1 have been tested in CHB patients and have shown promising results. However, the dose of antibodies administered were significantly lower in CHB patients than in cancer patients to minimize immune related adverse events. Compared with antibodies, subcutaneously (SC) delivered siRNA have a short half-life in plasma and exposure is mainly concentrated in the liver, thereby reducing the potential for systemic toxicities. We have developed a GalNAc conjugated human PD-L1 (hPD-L1) siRNA lead molecule, ALG-072585, that demonstrated significant reduction of AAV-HBV infected hepatocytes through immune activation in human PD-1/PD-L1 double knock in (KI) mice while showing no signs of liver toxicity in uninfected mice of the same background.

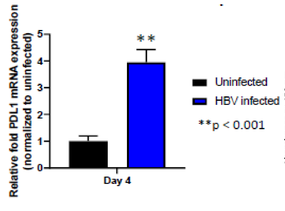
METHODS

siRNAs were synthesized on a MerMade synthesizer. In vitro human PD-L1 knockdown was evaluated in the SNU-387 cell line by RT-qPCR. GalNAc PD-L1 siRNA pharmacodynamics (PD) were studied by assessing Poly IC induced human PD-L1 levels in double KI mice. In double KI mice chronically infected with AAV-HBV, ALG-072585 and its parental siRNA, ALG-072571, were dosed SC as single agents (QW or QOW) or in combination with the HBV siRNA ALG-125819. Serial serum collections were tested for HBsAg, HBeAg, HBV DNA and ALT. Terminal serum samples were assayed for anti-HBsAg antibody. Mouse livers were stained for HBcAg, HBsAg and CD3 using IHC and scored for positive cells.

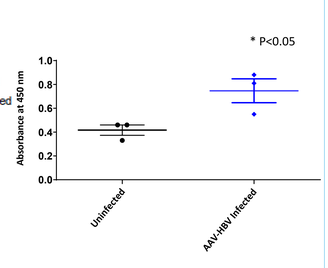
RESULTS

hPD-L1 was elevated in HBV preclinical models

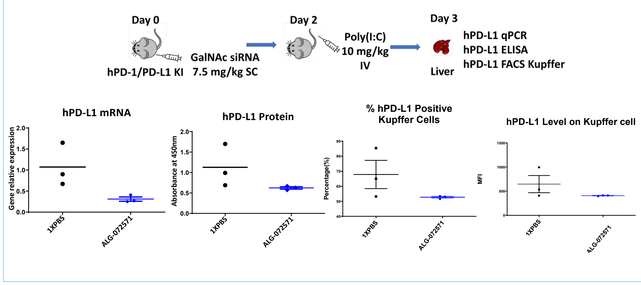
hPD-L1 RNA in QUAD 3-D Human Liver System PHH, LSEC, HSC and Kupffer Cells



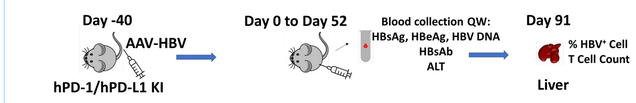
hPD-L1 Protein Levels in hPD-1 /hPD-L1 KI Mouse Livers



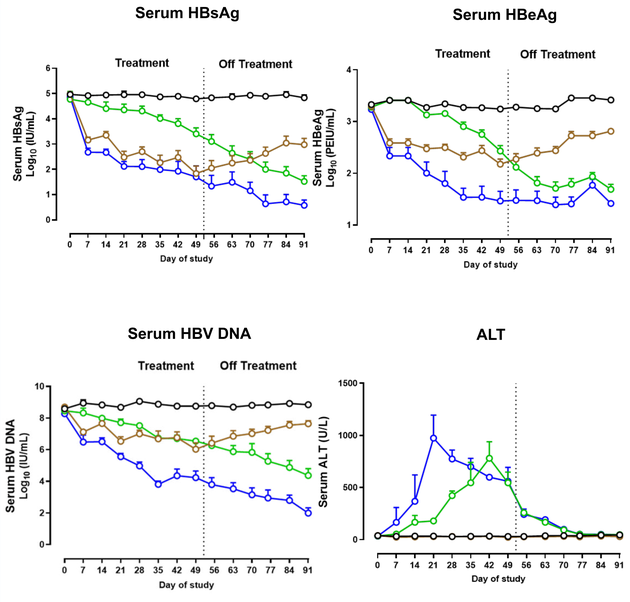
Study #1: Parental siRNA ALG-072571 reduced hPD-L1 in a double KI mouse PD study



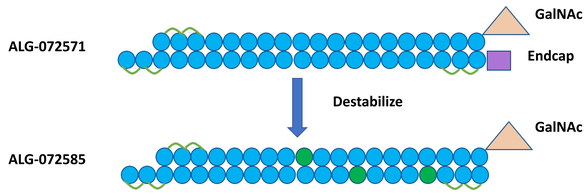
Study #2: ALG-072571 reduced HBsAg, HBeAg, HBV DNA and showed synergy with HBV siRNA in AAV-HBV double KI mice



O Vehicle Control
O HBV siRNA ALG-125819 4X5mg/kg QOW
O hPD-L1 siRNA ALG-072571 8X7.5 mg/kg SC QW
O ALG-072571 8X7.5 mg/kg SC QW + ALG-125819 4X5mg/kg SC QOW

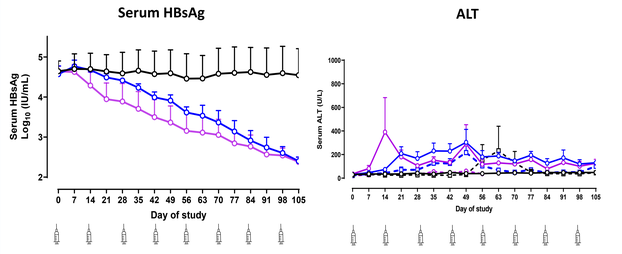


hPD-L1 siRNA ALG-072585 was generated by destabilizing ALG-072571



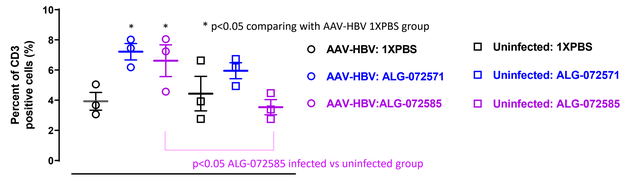
Study #3: ALG-072585 significantly improved the therapeutic window in double KI AAV-HBV mice

- AAV-HBV Double KI Mice: 1XPBS SC QOW
- AAV-HBV Double KI Mice: ALG-072571 7.5 mg/kg SC QOW
- AAV-HBV Double KI Mice: ALG-072585 7.5 mg/kg SC QOW
- Uninfected Double KI Mice: 1XPBS SC QOW
- Uninfected Double KI Mice: ALG-072571 7.5 mg/kg SC QOW
- Uninfected Double KI Mice: ALG-072585 7.5 mg/kg SC QOW

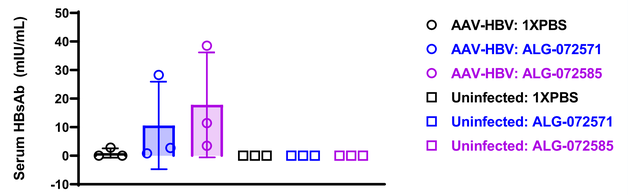


- Parent ALG-072571 showed higher level of ALT in AAV-HBV infected than uninfected double KI mice.
- ALG-072585 showed no ALT in uninfected double KI mice.

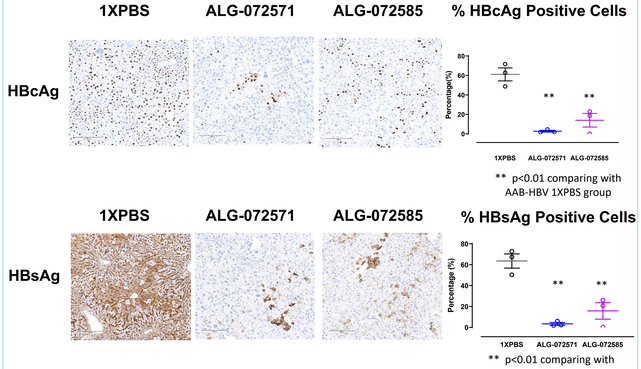
Study #3: ALG-072585 treatment induced CD3+ T cell increases in the livers of AAV-HBV infected double KI mice



Study #3: Individual AAV-HBV mice in the ALG-072585 treated group showed elevated serum HBsAb



Study #3: ALG-072585 treatment showed significant reduction of HBV-infected hepatocytes in liver IHC



CONCLUSIONS

1. hPD-L1 was elevated in HBV-infected 3-D liver chips and the livers of AAV-HBV infected hPD-1/hPD-L1 double KI mice.
2. Parental hPD-L1 siRNA ALG-072571 reduced hPD-L1 in the livers of poly(I:C) treated hPD-1/hPD-L1 double KI mice.
3. In AAV-HBV double KI mice, ALG-072571 reduced serum HBsAg, HBeAg and HBV DNA 3.3 $\log_{10}$ IU/mL, 1.73 $\log_{10}$ PEIU/mL and 4.5 $\log_{10}$ IU/mL respectively as a single agent; 4.3 $\log_{10}$ IU/mL, 2.0 $\log_{10}$ PEIU/mL and 6.9 $\log_{10}$ IU/mL respectively when combined with an HBV siRNA.
4. Lead optimization of ALG-072571 by chemical destabilization yielded ALG-072585 which retained in vivo potency of its parent in reducing infected hepatocytes and serum HBsAg but did not induce ALT in uninfected double KI mice.
5. In AAV-HBV infected double KI mice, ALG-072585 increased T cell infiltration in the liver and induced anti-HBsAg antibodies in the blood.

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Financial disclosure: All authors are current or former employees of Aligos Therapeutics, Inc.