Hepatitis B Surface Antigen (HBsAg) is Significantly Reduced by the HBV small interfering RNA (siRNA) ALG-125755 in HBV-Infected Cells and the AAV-HBV Mouse Model

Megan E. Fitzgerald*

Jin Hong, Saul Martinez Montero, Hua Tan, Tilani De Costa, Yuchun Nie, Rajendra Pandey, Vivek K. Rajwanshi, Aneerban Bhattacharya, Cheng Kao, Hyunsoon Kang, Min Luo, Elen Rosler, Sucheta Mukherjee, Kusum Gupta, David B. Smith, Lawrence M. Blatt, Julian A. Symons, Leonid N. Beigelman

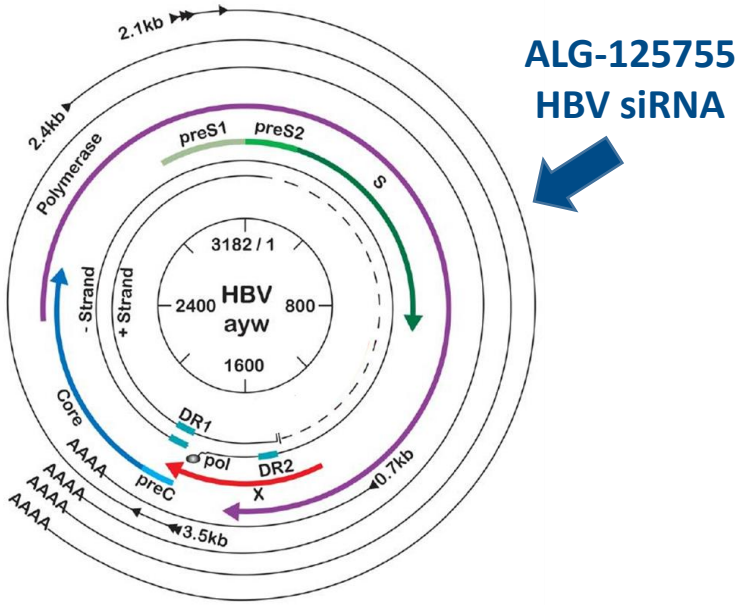
Aligos Therapeutics, Inc.

1 Corporate Dr. South San Francisco, CA 94080

*Presenting author

The GalNAc-Conjugated siRNA ALG-125755 Targets a Highly Conserved Region of the HBV Genome

ALG-125755 targets the small HBsAg open reading frame



- **ALG-125755 exhibits pan-genotypic, broad coverage via predicted high-affinity binding across different HBV genotype sequences**
- Predicted binding free energy (ΔG) for perfect match target sites = -28.5 kcal/mol
- 95% of HBV genomes Genotype A-J have 0 mismatches (MM) to ALG-125755, and the remaining 5% have 1 MM

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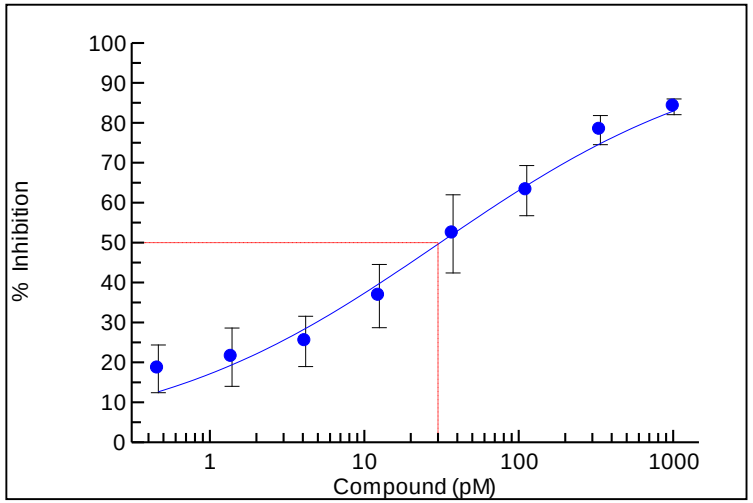
ALG-125903, the Parent Unconjugated siRNA, Demonstrates Potent Inhibition of HBsAg Release in Multiple Cell Lines



Cell Line	EC_{50} (pM)	CC_{50} (pM)
HepG2.2.15[a]	10.7	>1000
HBV-infected PHH[b]	28.8	>50,000

- Unconjugated siRNAs were transfected using Lipofectamine® RNAiMAX
- The secreted HBsAg was quantified by ELISA and cell viability was assessed using Cell Titer Glo

- **ALG-125903 exhibits picomolar EC_{50} values for inhibition of HBsAg release in HepG2.2.15 and HBV-infected PHH cells**
- **Cytotoxicity is not observed up to the highest concentrations tested**

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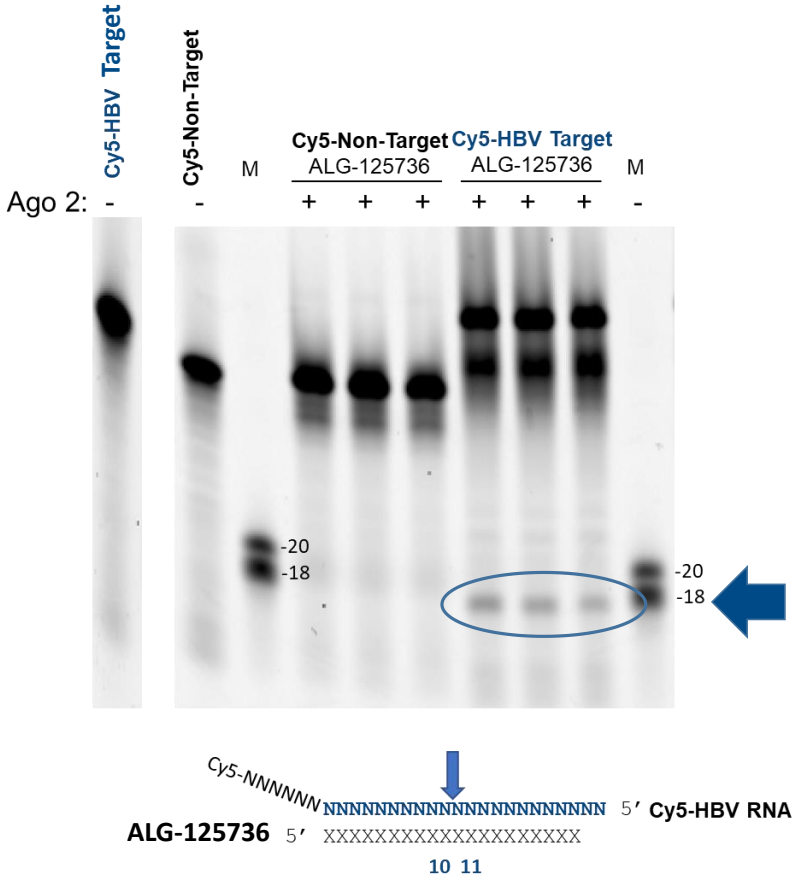
[a] A commonly used HBV model that contains integrated Genotype D HBV genomes.
[b] The PHH cells are infected at 200 MOI (ge) 4 days prior to siRNA transfection with live genotype D laboratory strains from HBV-infected cells.

Confirmation of ALG-125755 Mechanism of Action Using an In Vitro Ago2 Cleavage Assay



- Ago2 degradation of the target S-region HBV RNA sequence induced by the antisense strand (AS) of ALG-125755, ALG-125736, was qualitatively measured:
  - Using purified human Ago2, cleavage of the target RNAs was measured in the presence of the AS of the siRNA using denaturing polyacrylamide gel electrophoresis (PAGE)

- **ALG-125736 induced Ago-2 mediated cleavage of the target HBV RNA between the nucleotides (nt) that anneal to nt 10 and 11 of ALG-125736 (indicated by the blue arrow).**

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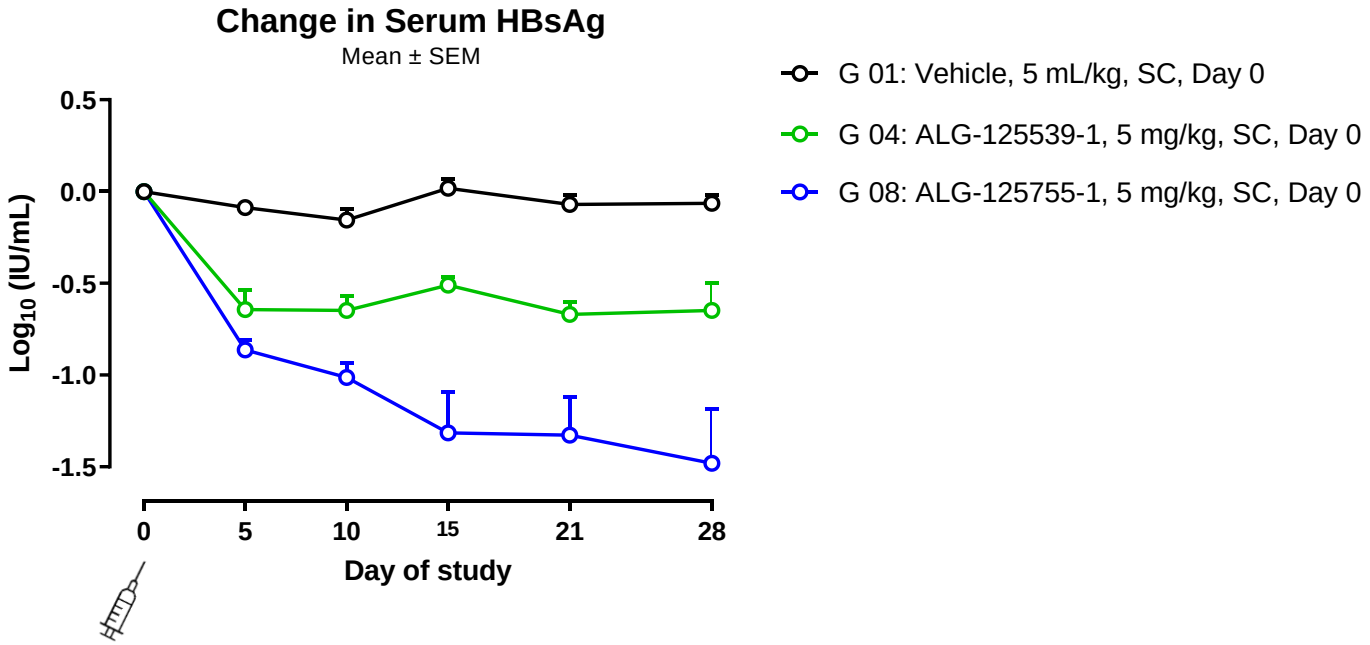
ALG-125755 Demonstrates Greater HBsAg Reduction in the AAV-HBV Mouse Model as Compared to an Unoptimized siRNA



- **A single, 5 mg/kg SC ALG-125755 dose reduces serum HBsAg by 1.5 $\log_{10}$ IU/mL from baseline**
- **ALG-125539 is an unoptimized siRNA that does not contain Aligos stabilizing chemistries and demonstrates a weaker reduction of HBsAg**

- ALT is not elevated in either ALG-125755 or ALG-125539 treated mice (data not shown)

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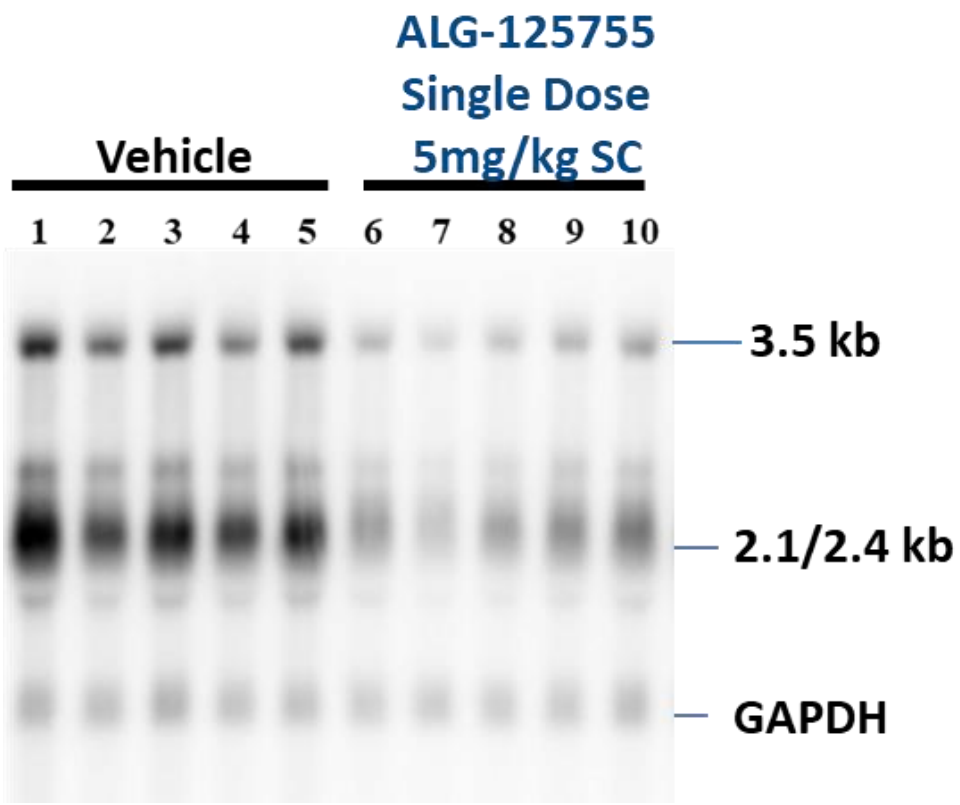
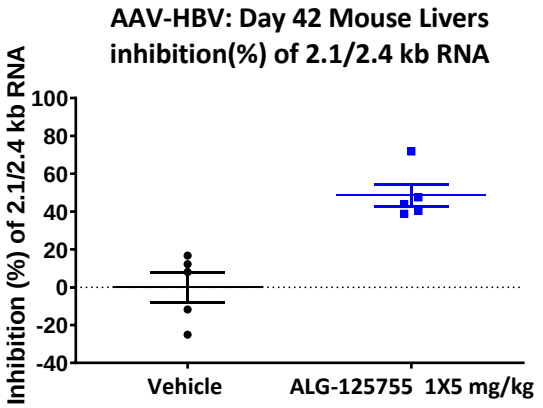
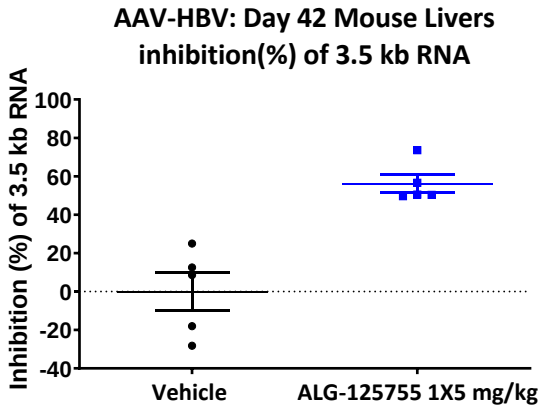
ALG-125755 Significantly Reduces HBV RNA Levels in AAV-HBV Mouse Liver







- A single, 5 mg/kg SC ALG-125755 dose reduces liver levels of HBV RNAs at Day 42
- Liver HBsAg is also reduced, with a corresponding serum HBsAg reduction of 1.25 $\log_{10}$ IU/mL

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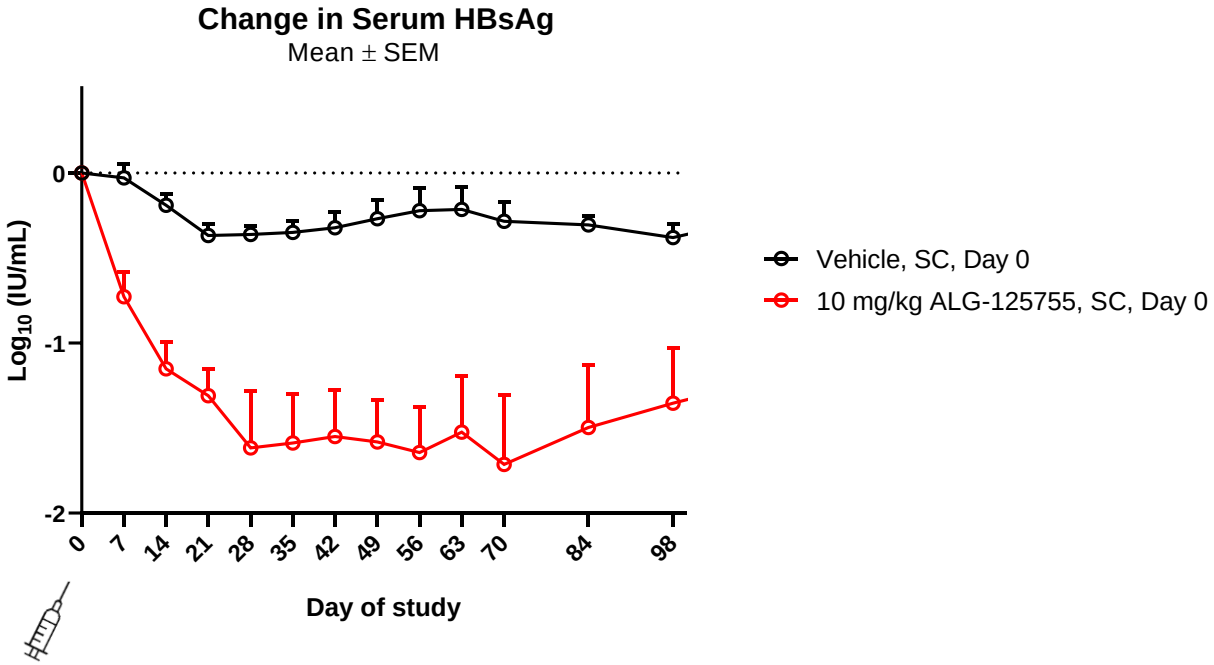
Durable and Sustained Reduction of Serum HBsAg by ALG-125755 in AAV-HBV Mice



- **A single, 10 mg/kg SC ALG-125755 dose decreased serum HBsAg by 1.7 $\log_{10}$ IU/mL from baseline**
- **Reduced HBsAg levels were maintained for 70 days post-dose**

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Results and Conclusions

- ALG-125755 significantly reduced HBsAg in multiple HBV-infected cell lines
- The ALG-125755 mechanism of action was confirmed with an *in vitro* Ago2 RNA cleavage assay
- Stabilization chemistry used in ALG-125755 improved HBsAg knockdown in vivo as compared to an unmodified siRNA
- ALG-125755 demonstrated significant and sustained HBsAg knockdown in the AAV-HBV mouse model with reductions of HBsAg levels observed for 70 days post-dose
- Northern blot analysis of HBV RNAs from AAV-HBV mouse livers showed decreased levels with ALG-125755 treatment, indicating on-target activity
- Further development of ALG-125755 is ongoing