

ALG-125755, a Small Interfering RNA (siRNA) Against Hepatitis B Virus (HBV) Effectively Inhibits Hepatitis B Surface Antigen (HBsAg) Secretion in HBV Cell Models and the AAV-HBV Mouse Model

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Background and Aims

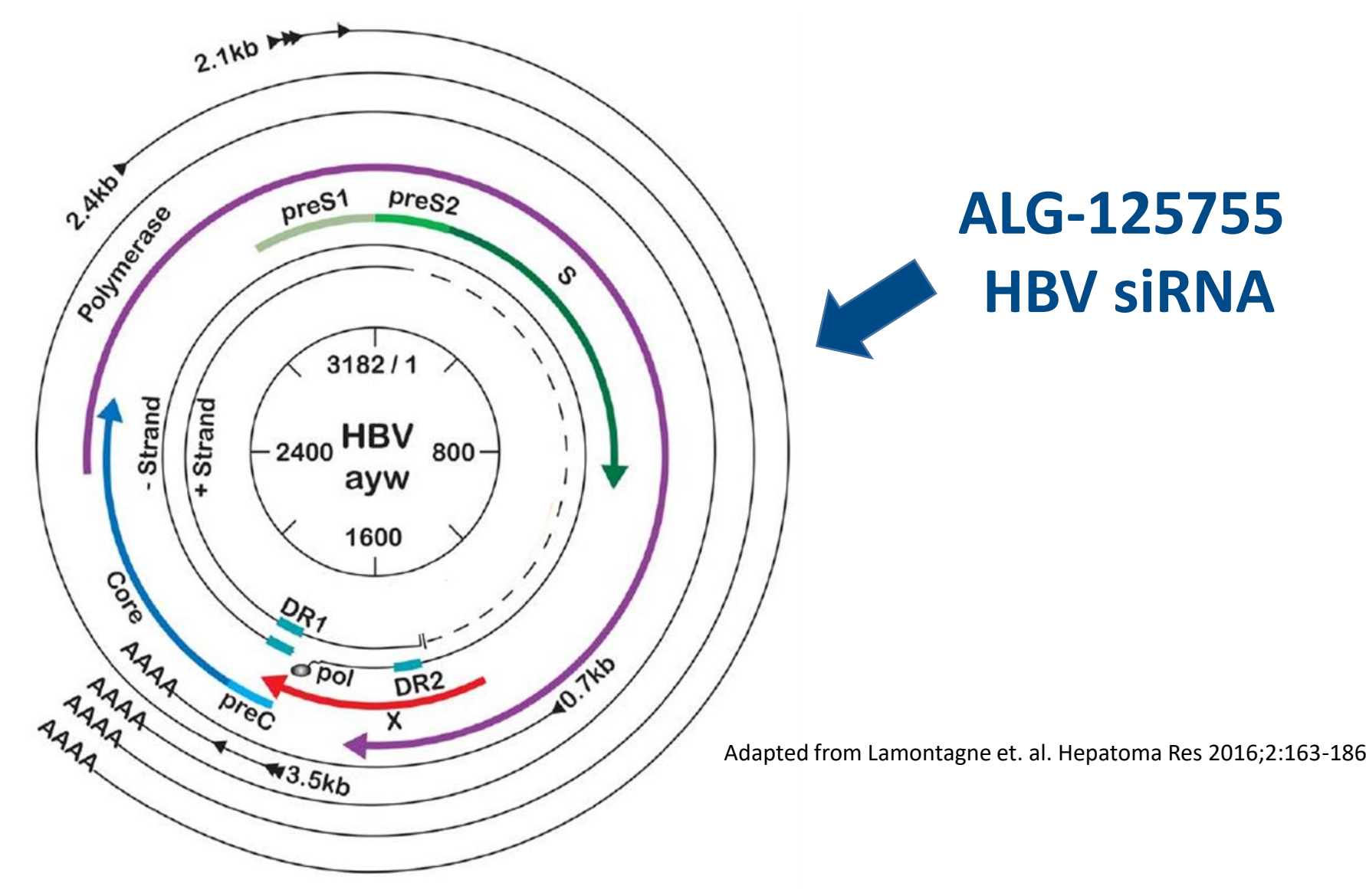
The current standard of care for chronic hepatitis B (CHB) fails to reduce HBsAg needed to attain a “functional cure”. HBV siRNA therapies have been shown to be effective in reducing HBsAg in CHB patients. In this study, we applied multiple stabilization chemistries to distinct HBV siRNA sequences and achieved significant improvements in depth and duration of HBsAg knockdown over their parent compounds in the serum of the AAV-HBV mouse model. ALG-125755, a proprietary GalNAc conjugated form of the siRNA ALG-125903 that utilizes these chemistries was selected for further development.

Methods

HBV siRNAs were profiled for HBsAg release in HepG2.2.15 cells, a commonly used genotype D HBV cell model, as well as a primary human hepatocyte (PHH) live HBV infection model. Unconjugated siRNAs were transfected using Lipofectamine® RNAiMAX. For PHH cells, infection with HBV at 200 MOI (ge) occurred 4 days prior to the transfection of siRNA. The secreted HBsAg was quantified by ELISA. The antisense strand of ALG-125755, ALG-125736 was analyzed for HBV RNA cleavage in the human Ago-2 biochemical assay. In the AAV-HBV mouse model, HBV siRNAs conjugated with GalNAc were administered subcutaneously (SC) with blood collections every 5 days for HBsAg and ALT assessments. Livers collected from the AAV-HBV study at the end of study were assayed for HBsAg ELISA and HBV RNA Northern blot. The immune activation profiles of ALG-125755 and its unconjugated form ALG-125903 were assessed by human PBMC cytokine release assays.

Results

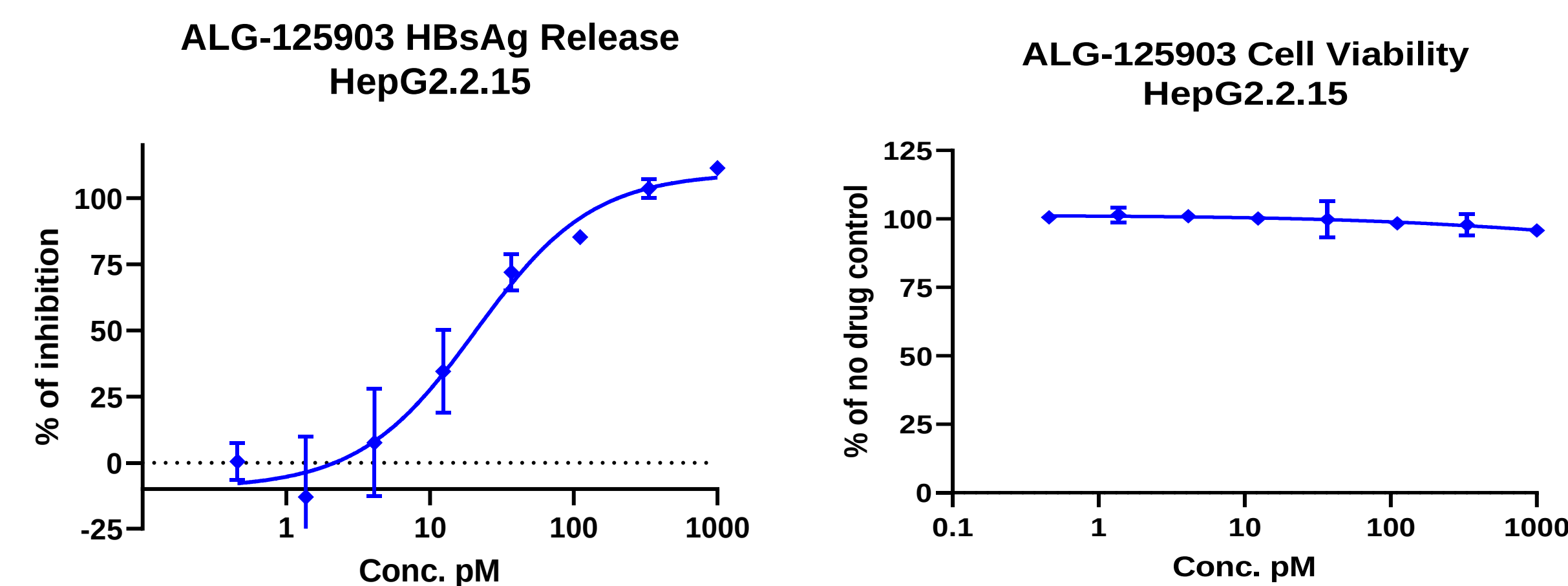
ALG-125755 Targets the ORF of Small HBsAg



Homology (0 or 1 Mismatch) Across HBV Genotypes

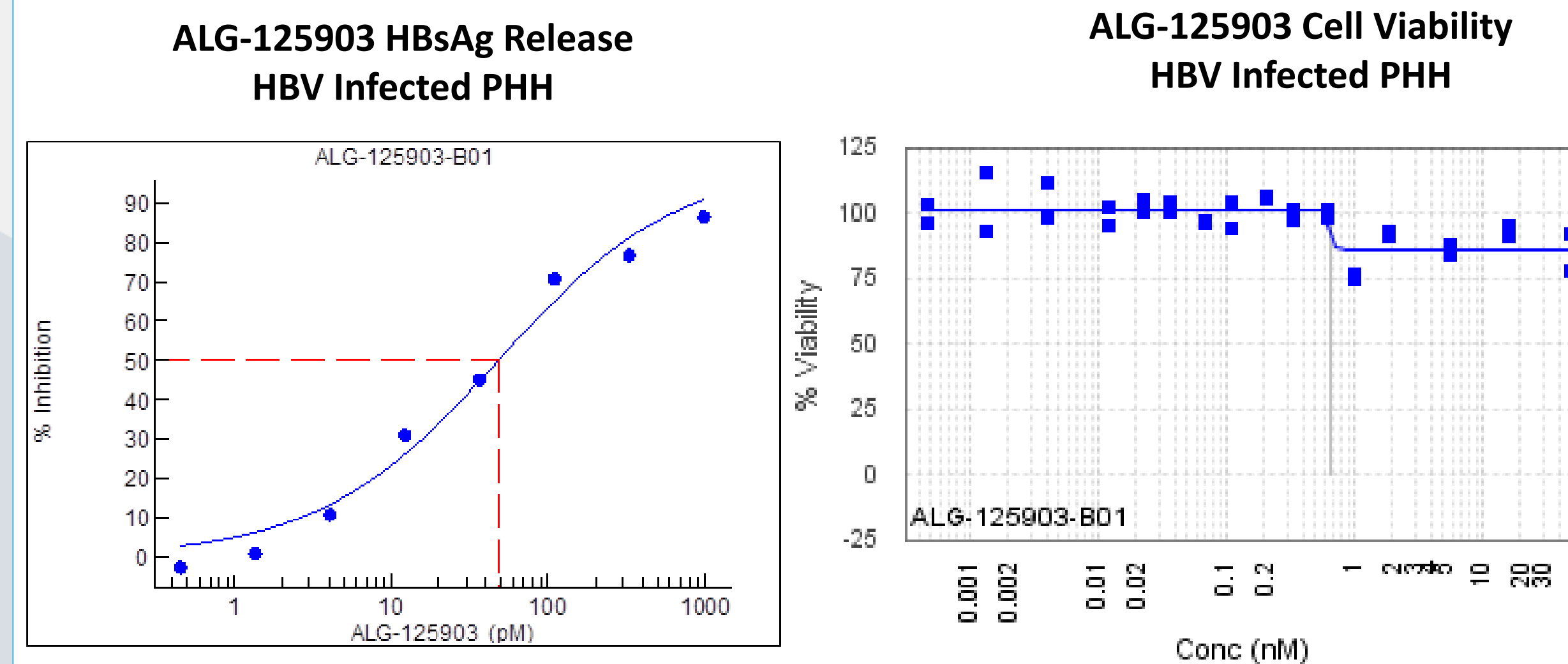
ID	Gt A	Gt B	Gt C	Gt D	Gt E	Gt F	Gt G	Gt H	Gt I	Gt J
ALG-125755	98.5%	99.5%	99.8%	99.6%	100%	99.7%	100%	100%	99.1%	100%

In Vitro Potency in HepG2.2.15



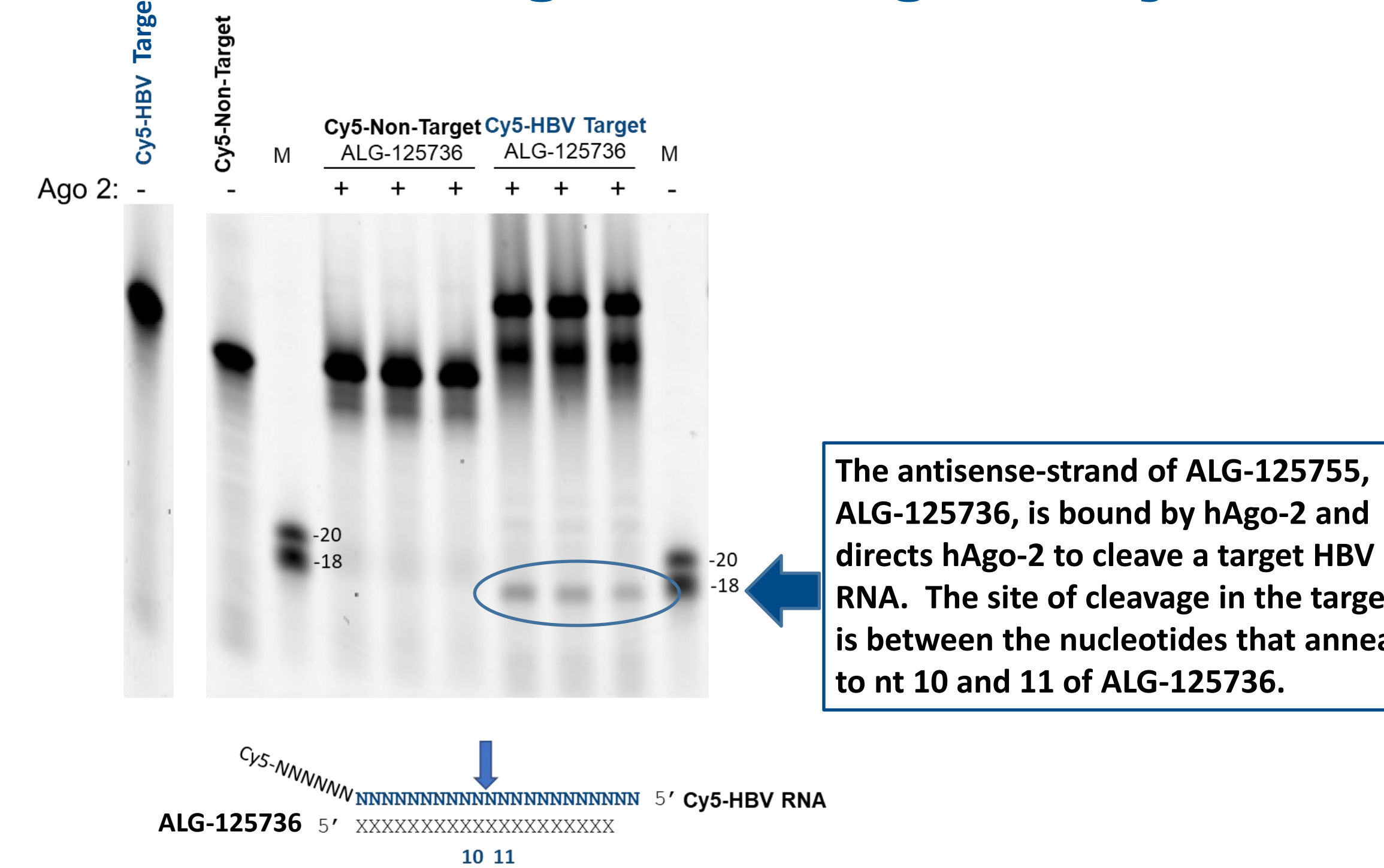
GalNAc Conjugated	Unconjugated	EC ₅₀ (pM)	CC ₅₀ (pM)
ALG-125755	ALG-125903	23.9 ± 5.8	>1000

In Vitro Potency in HBV Infected PHH



GalNAc Conjugated	Unconjugated	EC ₅₀ (pM)	CC ₅₀ (pM)
ALG-125755	ALG-125903	28.8	>50,000

Human Ago-2 Cleavage Assay



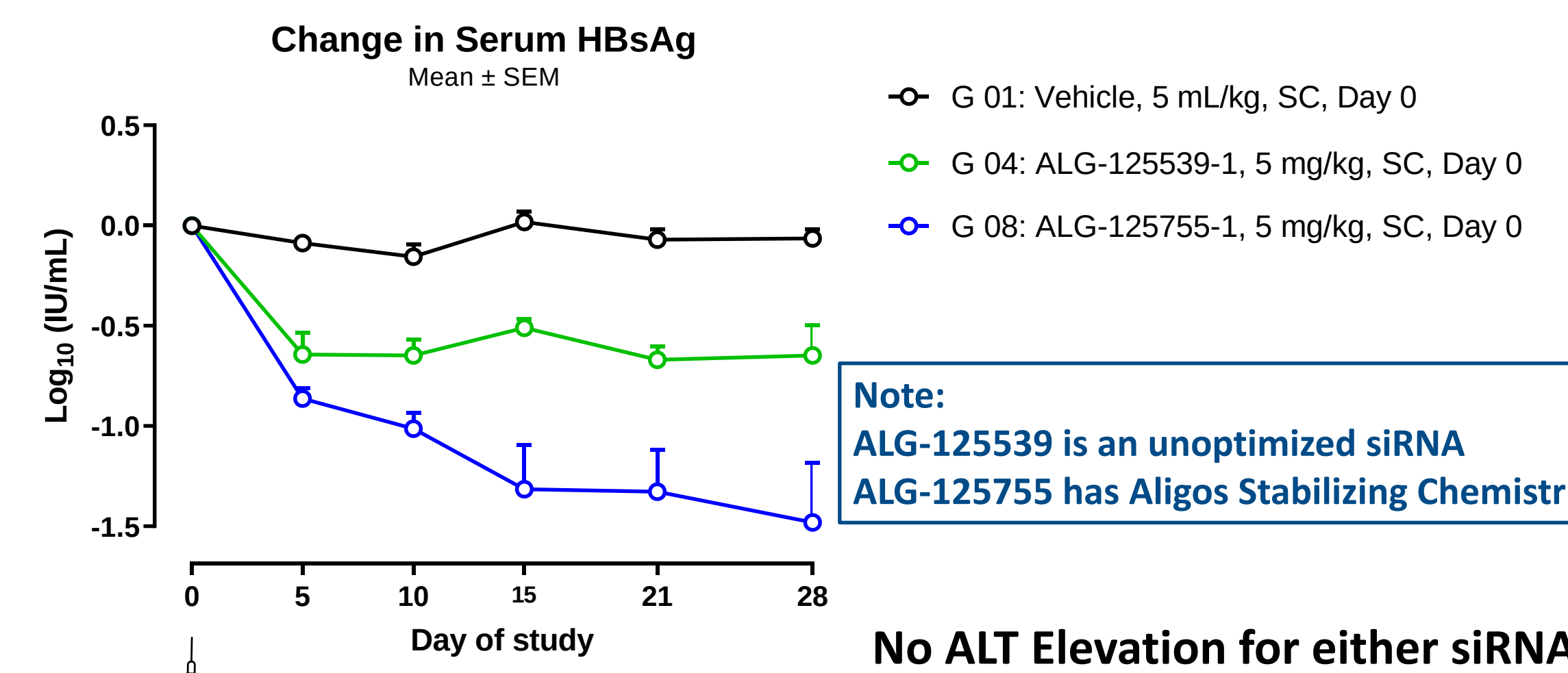
Good Safety in Multiple Cellular Systems

Hepatoma Cell Line	SNU387 CC ₅₀	SNU398 CC ₅₀
ALG-125903 Analog	>1000 nM	>1000 nM

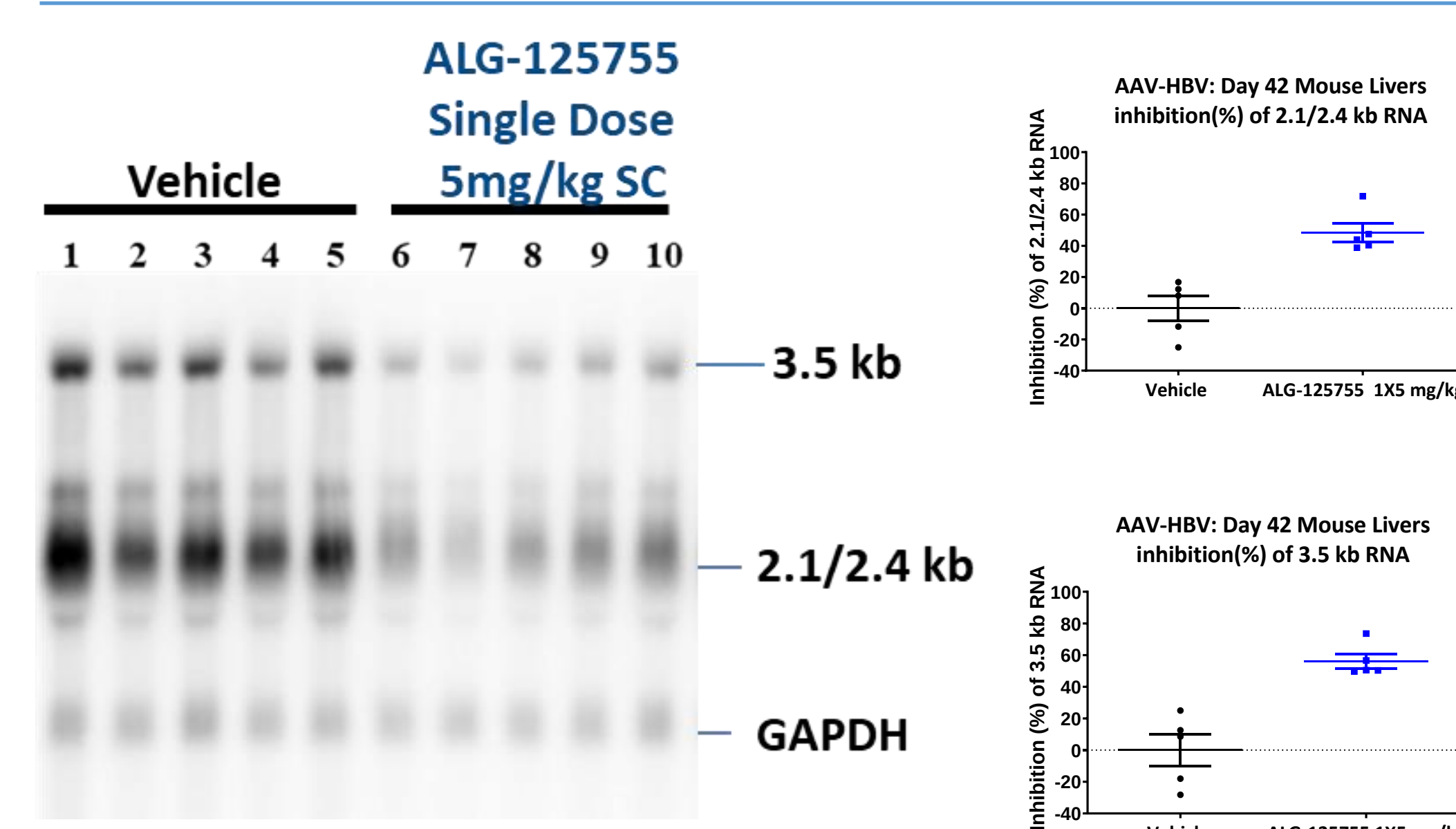
PHH Donor	HU18531 CC ₅₀	Hu8305 CC ₅₀	HYK CC ₅₀
ALG-125903	>500 nM	>500 nM	>500 nM

RNAseq Analysis:
Clean ALG-125903 Off Target Profile in HepG2.2.15

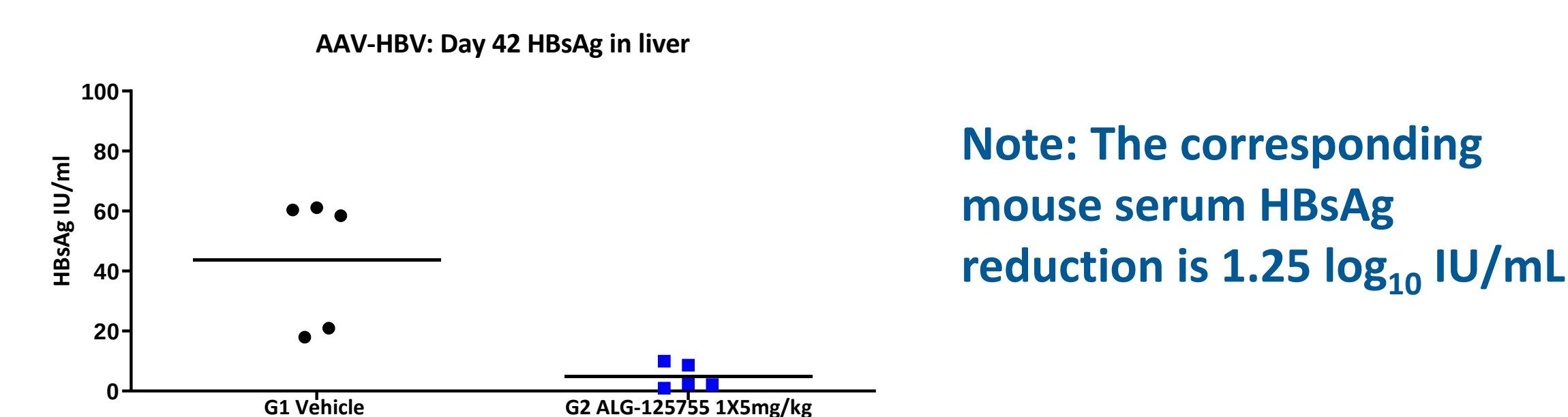
AAV-HBV: Improvement of In Vivo Potency with Aligos Stabilizing siRNA Chemistries



AAV-HBV: End of Study (Day 42) Analysis of Mouse Livers Following Single Dose of ALG-125755

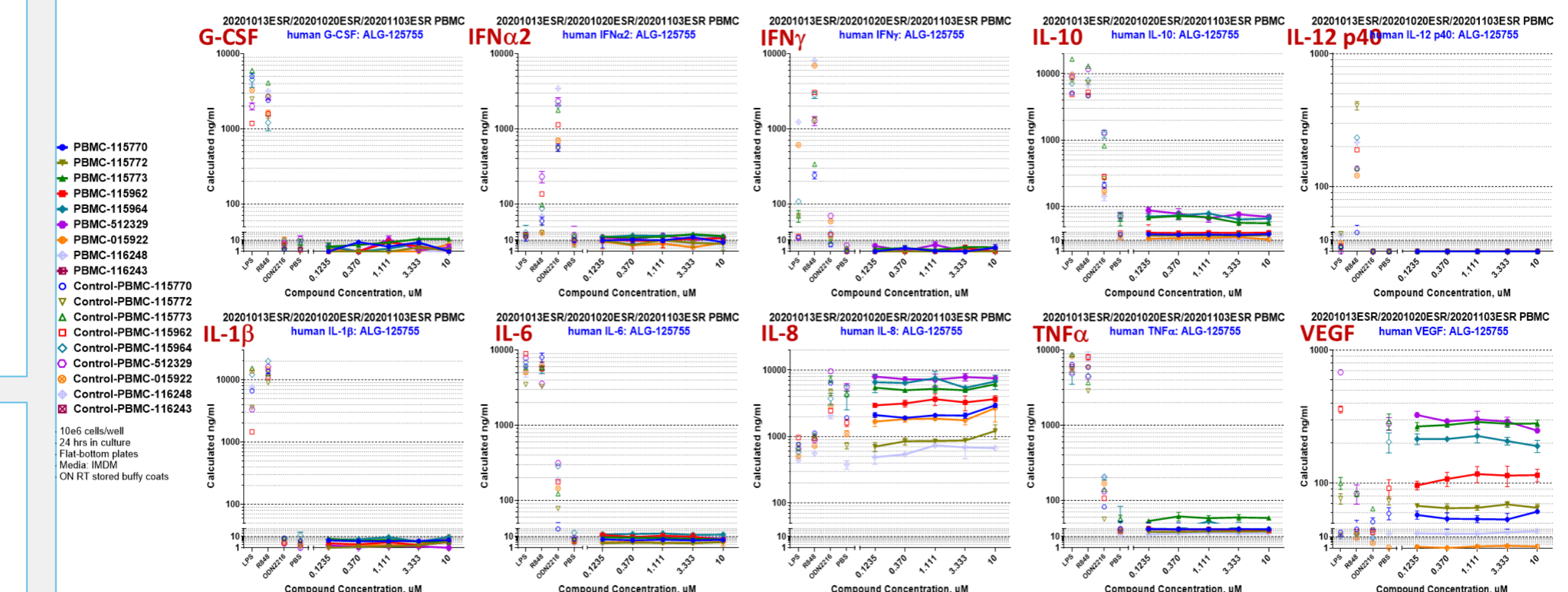


ELISA



ALG-125755: No Cytokine Induction in a Human PBMC Assay

• ALG-125755: PBMC from 9 Donors



• In addition, ALG-125903: Unconjugated siRNA Exhibited No Cytokine Induction

Summary of Results

- ALG-125755 targets the Small HBsAg ORF
- Targeted region has ≥99% homology across HBV genotypes
- 23.9 and 28.8 pM EC₅₀ in HepG2.2.15 and PHH assays respectively
- AAV-HBV mouse model: 1.5 log₁₀ IU/mL serum HBsAg knockdown with a single dose of 5 mg/kg, SC
- Good in vitro safety profile in multiple cellular systems
- No cytokine induction in the human PBMC Assay
- Demonstrated MOA : through Ago-2 associated HBV RNA knockdown
- Demonstrated favorable combination effects when combined with other modalities (1).

Conclusions

The HBV siRNA, ALG-125755, demonstrated good potency and safety profiles in HBV cellular and animal model systems. The compound is currently being developed as potential treatment for Chronic Hepatitis B.

Reference

- Hua Tan, Jin Hong, Hyunsoon Kang, Min Luo, Yuchun Nie, Suping Ren, Rajendra Pandey, John Cortez, Vivek K. Rajwanshi, David B. Smith, Lawrence M. Blatt, Leonid N. Beigelman and Julian A. Symons Combination drug interactions of hepatitis B virus (HBV) small interfering RNA (siRNA) and antisense oligonucleotides (ASO) in vitro and in vivo EASL 2021 Abstract # 1257

Financial Disclosures

All authors are employees of Aligos Therapeutics, Inc.