Lead Optimization and Selection of a Potential Best-in-Class HBV ASO

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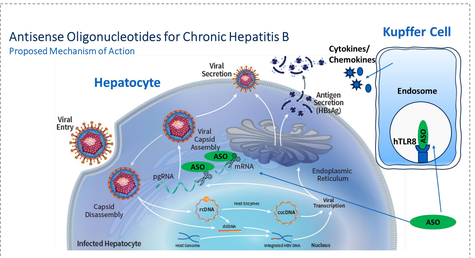
BACKGROUND

To achieve functional cure in chronic hepatitis B, the future treatment regimen will likely include direct antiviral and immunomodulatory drugs. Current clinical stage HBV ASOs, bepirovirsen (GSK-836)[1, 2] and AHB-137[3], are unique in their dual RNase H-mediated direct antiviral and immunomodulatory (e.g. TLR8 agonist) activities. GSK-836 as a single agent achieved a moderate functional cure rate of ~10% in a phase 2b trial; this was superior to many other modalities such as siRNAs, but still suboptimal for a functional cure rate. Our aim is to develop a best-in-class HBV ASO with an improved overall therapeutic index over both clinical stage HBV ASOs.

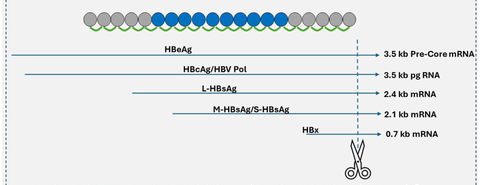


METHODS

RNase H-mediated activity was analyzed with an HBsAg ELISA in HepG2.2.15 cells and AAV-HBV mice. To validate ASO hTLR8 activity in vivo, hTLR8 knock-in mice were dosed with ASO subcutaneously and mouse cytokine induction was monitored. Toxicity derived from ASO off-target effects was measured in a HepG2 Caspase3/7 assay and an ATP assay in InSphero™ 3-D human liver microtissues. PXB mice with humanized livers were tested to assess the potential hepatotoxic effect of the ASOs.

RESULTS

ASO Platform with Novel Monomer



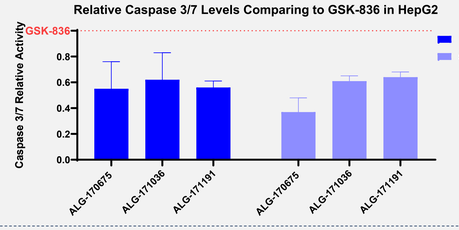
ALG-170675, ALG-171036 and ALG-171191 derived from the novel monomer platform target all HBV transcripts

RESULTS

Novel Chemistries Resulted in Lower T_m, Less Off-target Activity

ASO ID	T_m for Duplex with RNA Target (°C)	HepG2.2.15 HBsAg Knock-Down EC_{50} (nM)	HepG2.2.15 CTG CC_{50} (nM)
GSK-836	58.1	6.5 ± 2.8	>167
ALG-170675	50.2	8.8 ± 1.8	>167
ALG-171036	53.5	2.5 ± 0.2	>167
ALG-171191	51.8	2.5 ± 0.4	>167

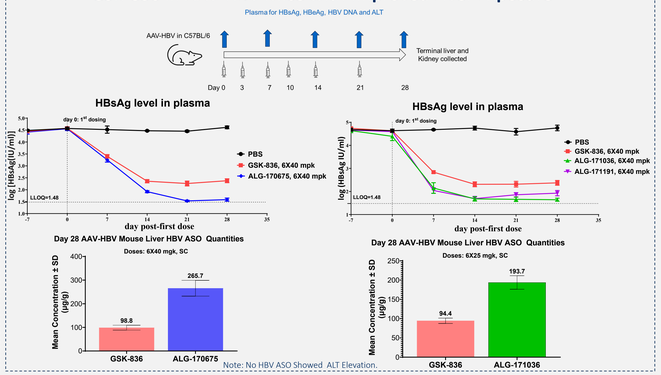
Caspase 3/7 Assay in HepG2 Cell Line



Lead ASOs Demonstrated TLR8 Agonist Activity with Minor TLR9 Activation in HEK-Blue TLR Cell Lines

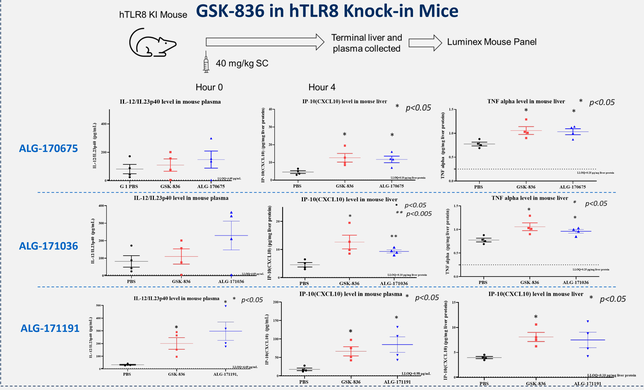
ALG#	hTLR7 Emax Fold vs. PBS	hTLR8 Emax Fold vs. PBS	hTLR9 Emax Fold vs. PBS	mTLR8 Emax Fold vs. PBS	Null Emax Fold vs. PBS
GSK-836	1.07	1.89	1.29	1.06	1.12
ALG-170675	1.03	1.87	1.18	1.06	1.07
ALG-171036	1.07	2.24	1.22	1.02	1.11
ALG-171191	1.30	2.18	1.14	1.06	0.99
R848 (TLR7/8)	3.71	6.15		1.04	1.01
R837 (TLR7)	1.66				1.01
GS9688 (TLR8)		7.30		1.00	1.03
ODN2006 (TLR9)			2.33		1.03

Lead ASOs Showed Improved RNase H Mediated In Vivo Activity Over GSK-836 in AAV-HBV Mice with Improved Liver Exposure

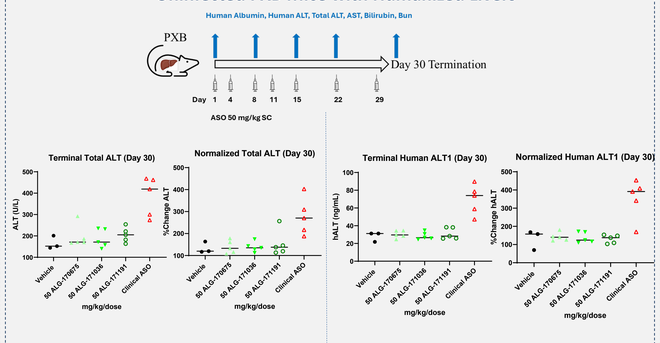


Note: No HBV ASO Showed ALT Elevation.

Lead ASOs Showed Similar Cytokine Induction Profiles to GSK-836 in hTLR8 Knock-in Mice

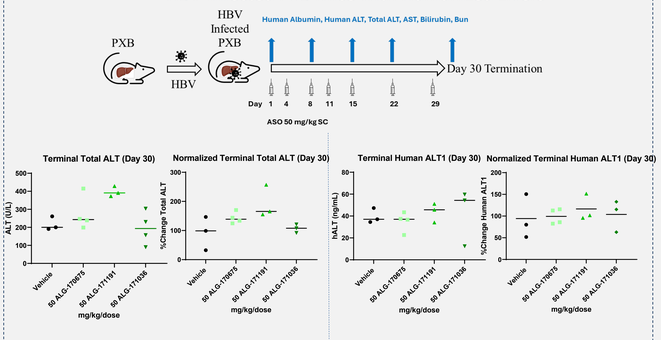


ALG-170675, ALG-171036 and ALG-171191 Exhibited No Liver Toxicity in Uninfected PXB Mice with Humanized Livers



Note: Clinical ASO is an ASO that showed hepatotoxicity in human clinical trial.

ALG-170675 and ALG-171036 Demonstrated No Liver Toxicity in HBV-Infected PXB Mice with Humanized Livers



CONCLUSIONS

- ALG-170675 and ALG-171036 are optimized HBV ASO leads that emerged as the top two candidates after PXB mouse studies.
- Both ASOs showed lower T_m and less in vitro cytotoxicity than GSK-836.
- Improved in vivo efficacy and liver exposure in AAV-HBV mice was observed when compared to GSK-836 with both ASOs.
- Similar immunomodulatory profiles as GSK-836 were observed in vitro and in vivo with both ASOs.
- No hepatotoxicity in uninfected or HBV-infected PXB mice with humanized livers was observed with either ASO.

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