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Next generation hepatitis B virus antisense oligonucleotides incorporating novel chemistries demonstrated significantly improved properties compared to current clinical candidates

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ALIGOS THERAPEUTICS

Background And Aims

Chronic hepatitis B (CHB) patients can achieve functional cure after monotherapy with antisense oligonucleotides (ASO) targeting hepatitis B virus (HBV) RNA, e.g., bepirovirsen (GSK-836) and AHB-137. This is a superior outcome compared to many other new anti-HBV agents that demonstrate no signs of HBV S antigen (HBsAg) loss. However, the reported rates of HBsAg loss at the end of ASO treatment have only been moderate and the relapse rates during the off-treatment follow up period are high. Our goal is to develop a best-in-class HBV ASO that improves the rate of functional cure and/or safety over current clinical compounds.

Aligos has developed an ASO platform utilizing novel monomers that could potentially reduce ASO toxicity and improve ASO liver to kidney ratio. After rigorous screening, 10 HBV ASOs emerged as lead candidates. ALG-170675 is one of the top performers among the lead candidates and showed favorable in vitro and in vivo profiles when compared with GSK-836.

Method

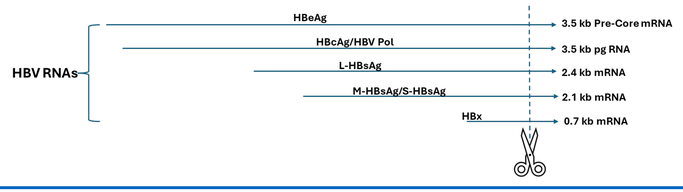
In vitro and in vivo RNase H mediated-activity was analyzed with an HBsAg ELISA assay in the supernatant of the HepG2.2.15 cell line or in the plasma of AAV-HBV mice. HEK-Blue hTLR8 and human peripheral blood mononuclear cell (PBMC) assays were used to monitor TLR8 activity. To validate ASO hTLR8 activity in vivo, hTLR8 knock-in (KI) mice were used as wild-type mice lack a functional TLR8 protein. Toxicity derived from ASO off-target effects was measured in a HepG2 Caspase3/7 assay. In AAV-HBV mice, plasma ALT was monitored to detect signs of drug related liver toxicity.

ALG-170675: Unconjugated ASO Targeting the X Region of HBV Transcripts

Aligos ASO Platform With Novel Monomer

ALG-170675

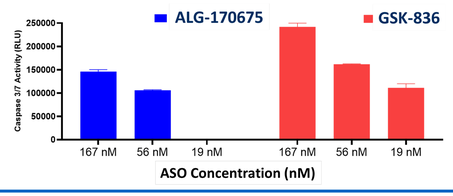
ALG-170675 mediated RNase H activity cleaves all 5 HBV RNAs and reduces all HBV proteins



ALG-170675 Exhibited a Lower T_m, Similar In Vitro RNase H Mediated Activity and Less In Vitro Toxicity Than GSK-836

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	8.1	>167
ALG-170675	50.2	9.2	>167

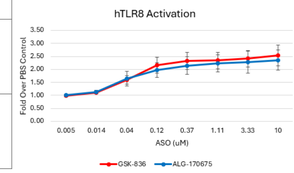
HepG2 Cell Caspase3/7 Assay



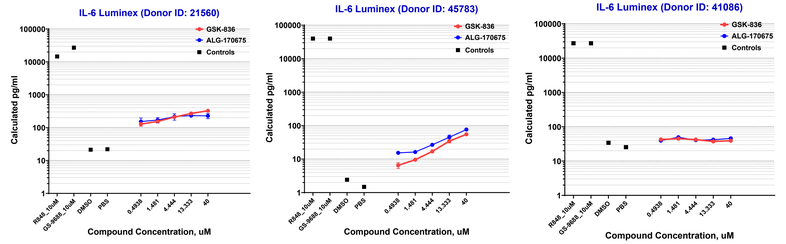
ALG-170675 Showed Similar Human TLR8 Agonist Activity As GSK-836 In Cell Based Assays

ASO	HEK-Blue-hTLR8	HEK-Blue-hTLR7	HEK-Blue-mTLR8	HEK-Blue Null
GSK-836 Emax Fold Over PBS	2.4 X	1.07 X	1.06 X	1.12 X
ALG-170675 Emax Fold Over PBS	2.3 X	1.03 X	1.06 X	1.07 X

HEK-Blue-hTLR8 Assay

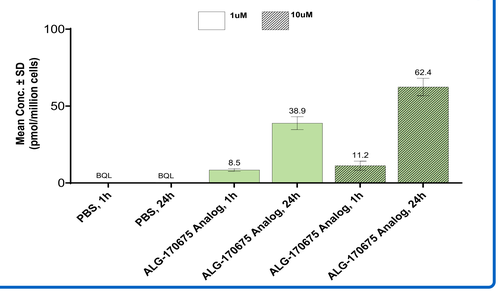


Human PBMC Assay



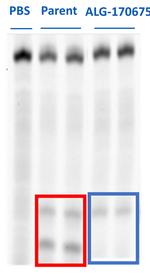
ALG-170675 Analog: In Vitro Macrophage Free Uptake

- Human primary CD14+ monocytes in 24-well plates were differentiated to M1 macrophages
- A significant amount of ALG-170675 analog was taken up by macrophages without transfection



ALG-170675 Biochemical Assays For MOA: Interaction With Human RNase H And Human TLR8

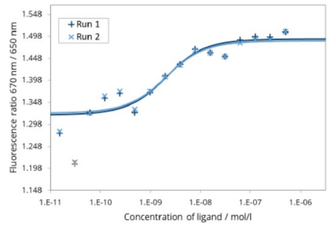
ALG-170675 mediated hRNase H cleavage of HBV RNA



HBV RNA Substrate

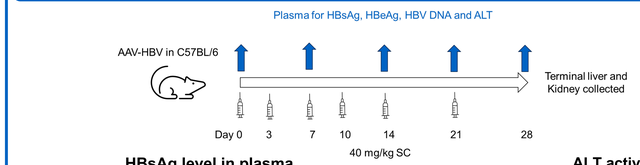
ALG-170675: Incorporation of a novel monomer altered the ASO cleavage pattern on the target RNA

ALG-170675 binds to hTLR8 protein

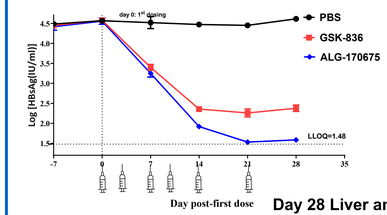


K_D = 1.58 nM

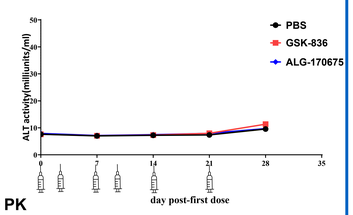
ALG-170675 Showed Improved PK and In Vivo Efficacy Compared to GSK-836 in the AAV-HBV Mouse Model



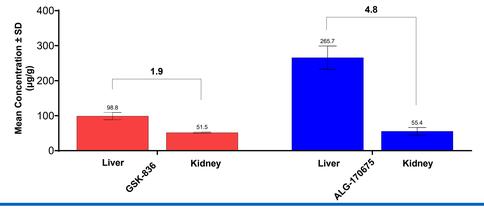
HBsAg level in plasma



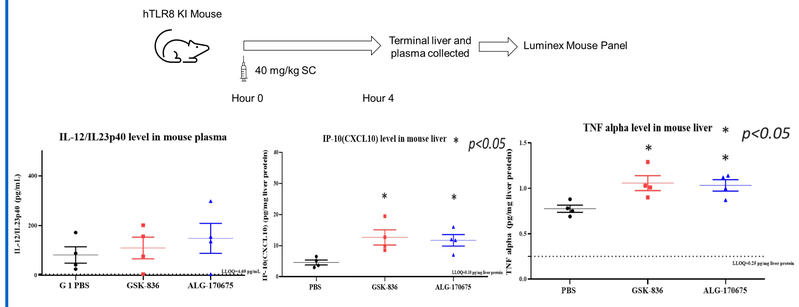
ALT activity in plasma



Day 28 Liver and Kidney PK



ALG-170675 Showed Similar Cytokine Induction as GSK-836 in Human TLR8 Knock-in Mice



Conclusions

1) Our novel monomer technology was applied to our 2nd Generation HBV ASO discovery program, resulting in the selection of 10 lead ASOs after screening
2) ALG-170675 is a lead candidate that showed an advantageous preclinical profile when compared with GSK-836 with better in vitro safety, enhanced liver exposure and liver to kidney ratio and improved in vivo efficacy
3) Similar hTLR8 agonist activity to GSK-836 was observed in vitro and in vivo
4) ALG-170675 demonstrated potential to be a best-in-class HBV ASO

Financial disclosure: all authors are current employees of Aligos Therapeutics Inc. or Amoytop Biotech.