BEST-IN-CLASS ANTISENSE OLIGONUCLEOTIDES AGAINST HEPATITIS B VIRUS: NEXT GENERATION BRIDGED NUCLEIC ACID CHEMISTRIES SIGNIFICANTLY INCREASE IN VIVO EFFICACY AND REDUCE HEPATOTOXICITY IN MICE

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In vitro screening of LNA ASOs was carried out in HepG2.2.15 cells using an HBsAg release assay. A potent LNA-containing ASO was chosen for adeno-associated virus (AAV)-HBV mouse model and exploratory mouse toxicity animal models, and patients with CHB. Hepatotoxicity is a major side effect of reducing HBsAg is key to achieve functional cure in chronic hepatitis B (CHB) patients. Antisense oligonucleotides (ASOs) are effective in reducing HBsAg in animal models, and patients with CHB. Hepatotoxicity is a major side effect of ASOs and is exacerbated with high affinity more active Locked-Nucleic Acid (LNA) modified ASOs. Next generation Bridged Nucleic Acid (BNA) monomers can reduce hepatotoxicity while maintaining efficacy. We have therefore utilized these BNA chemistries in our LNA-containing hepatitis B virus (HBV) targeting ASOs.

Methods

Background

High genotypic coverage for Hepatitis B virus (HBV) modified ASOs. Next generation Bridged Nucleic Acid (BNA) monomers can reduce hepatotoxicity while maintaining efficacy. We have therefore utilized these BNA chemistries in our LNA-containing hepatitis B virus (HBV) targeting ASOs.

Potency and In Vitro Cytotoxicity of ASOs with Same Sequence

<table>
<thead>
<tr>
<th>ASO</th>
<th>$iC50$ (µM)</th>
<th>$CC50$ (µM)</th>
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<tbody>
<tr>
<td>ALG-021467</td>
<td>0.28</td>
<td>&gt;100</td>
</tr>
<tr>
<td>ALG-020576</td>
<td>2.24</td>
<td>&gt;100</td>
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- Potency of unconjugated ASO maintained and cytotoxicity reduced with chemical modifications
- In vitro potency of GalNAc conjugated ASO (ALG-020576) was lower, but effectively delivers the more potent unconjugated ASO (ALG-021467) to the target hepatocytes

No Liver Toxicity Observed with Weekly or Bi-Weekly ALG-020576 Dosing

- ALG-020576 has >95% homology across all HBV genotypes and low off-target potential
- Proprietary BNA chemical modifications of the same sequence showed that the potency was maintained while improving viability compared to the LNA- and DNA-containing parent
- In the AAV-HBV mouse model, ALG-020576 demonstrated a dose-responsive reduction in HBsAg and could achieve maximal HBsAg knockdown ≤ 1.5 log$_{10}$ IU/mL with no increase of ALT
- In mouse exploratory toxicity studies, LNA- and DNA-containing parent at 30 mg/kg/doe (with or without GalNAc conjugation) demonstrated elevations of ALT/AST, along with microscopic changes consisting of hepatocellular apoptosis/necrosis indicative of liver toxicity
- When BNA chemical modifications were made to the LNA- and DNA-containing parent (with or without GalNAc conjugation), no increases in ALT or AST were observed, and no adverse microscopic changes were noted in the liver at the same 30 mg/kg/doe level
- ALG-020576 was further profiled and demonstrated no liver toxicity up to 50 mg/kg/doe when dosed either weekly (3 doses) or bi-weekly (5 doses) for 2 weeks
- High liver concentrations of the potent unconjugated parent ALG-021467 were observed when dosed with ALG-020576
- Conjugation of the proprietary GalNAc also demonstrated favorable liver partitioning over kidney, particularly with weekly dosing

Conclusions

We demonstrated that applying next generation BNA and nucleobase chemistries in LNA ASO gapmers can significantly reduce in vivo hepatotoxicity and improve the therapeutic index. These results suggest that application of these novel nucleotide chemistries could lead to best-in-class anti-HBV ASOs.

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