ALG-010133, a Representative 5-Antigen Transport-inhibiting Oligonucleotide Polymer (STOPS™) Effectively Inhibits Hepatitis B Surface Antigen (HBsAg) Secretion in Multiple Hepatitis B Virus (HBV) Cell Models

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Background

CHRONIC Hepatitis B (CHB) is a global public health problem, affecting 300 million people. Current standard of care is highly efficacious in suppressing viral replication but fails to reduce HBsAg that suppresses the human immune system and prevents the attainment of “functional cure.” Nucleic acid polymers (NAs) such as REP-2139 (ALG-010004) significantly reduce circulating HBsAg in CHB patients when given as monotherapy and in combination therapy. We have studied oligonucleotides that can inhibit HBsAg secretion and have identified STOPS™ that share structural similarity with NAs but contain several novel chemical features. Here, we report the HBsAg inhibitory activity in multiple HBV cell models by ALG-010133, the leading STOPS molecule currently in Phase 1 clinical development.

Materials & Methods

STOPS were synthesized on ABI 394 and Expedite 8909 synthesizers using standard phosphoramidite chemistry. Compounds were profiled in the HepG2.2.15, PLC/PRF/5, HepG2-GNA, HepG2-GAl and HepG2-GB, compounds were administered by transfection using Lipofectamine RNAiMAX and secreted HBsAg was measured by ELISA 6 days post transfection. HepG2-NTCP cells and PBMC cells were infected with live HBV at 200 nM and STOPS were transfected five days later. The secreted HBsAg was quantitated by ELISA on day 6 post treatment. The intracellular HBsAg (HepG2.2.15) was measured by Western blot. PBMC were treated with test articles and controls for 24 hours. Cytokines (IL-6, IL-8, IL-10, IL-16, IL-22, IFN-γ, TNFα) were measured on IntelliCyt iQue Screen and analyzed using FlowCyt analysis software. The cytokines (IFNα) were tested by standard ELISA.

In vitro combination studies, a checkerboard design was used for dosing drugs in HepG2.2-15 and MacSynergy software was used to analyze the results.

Results

STOPS Inhibit HBsAg Release in the HepG2.2.15 Cell Model

The activity of STOPS in HepG2.2-15 cell model was determined using two different models.

1. **Summary:**
   - **STOPS Inhibit HBsAg Release in the HepG2.2.15 Cell Model**
   - **Compound Class Synergy**
   - **Volume (µM²%)**
   - **Synergistic/Antagonistic Interaction**

   - **STOPS:** ASO NUC CAM
   - **Synergy Volume (µM²) Synergistic/Antagonistic Interaction**
   - **Activity:**
     - **ALG-010133**
     - **ALG-020007**

   - **Conclusion:**
     - ALG-010133 is a representative STOPS molecule, potent with effective concentration reducing HBsAg release in the single-digit nM range in multiple HBV cell models.

Conclusions

1. STOPS are a class of oligonucleotides that can effectively inhibit HBsAg secretion.
2. ALG-010133, a representative STOPS molecule, is potent with effective concentrations reducing HBsAg release in the single digit nM range in multiple HBV cell models.
3. STOPS reduce intracellular HBsAg resulting in reduction of HBsAg secretion in the supernatant.
4. ALG-010133 does not activate proinflammatory cytokines in human PBMC assays.
5. ALG-010133 showed additive to synergistic effects when combined with other anti-HBV agents.
6. ALG-010133 is currently in Phase 1 clinical development.

References: