Poster # 0821

ALG-010133, a Representative S-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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ALIGOS THERAPEUTICS

Background

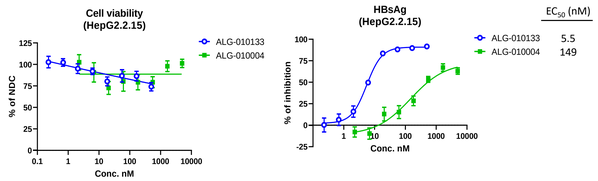
Chronic Hepatitis B (CHB) is a global public health problem, affecting 300 million people. Current standard of care is highly effective in suppressing viral replication but fails to reduce HBsAg that suppresses the human immune system and prevents the attainment of "functional cure". Nucleic acid polymers (NAPs) such as REP-2139 (ALG-010004) significantly reduce circulating HBsAg in CHB patients when given as monotherapy[1] and in combination therapy[2]. We have studied oligonucleotides that can inhibit HBsAg secretion and have identified STOPS™ that share structural similarity with NAPs 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-GtA, HepG2-GtB cell, HepG2-NTCP and primary human hepatocyte (PHH) live HBV infection system. In HepG2.2.15, PLC/PRF 5, HepG2-GtA and HepG2-GtB, compounds were administered by transfection using Lipofectamine RNAiMAX and secreted HBsAg was measured by ELISA 6 days post transfection. HepG2-NTCP cells and PHH cells were infected with live HBV at 200 moi (ge) and STOPS were transfected five days later. The secreted HBsAg was quantitated by ELISA on day 6 post-treatment. The intracellular HBsAg (HepG2.215) was measured by Western blot. PBMC were treated with test articles and controls for 24 hours. Cytokines (GM-CSF, IL-1β, IL-2, IL-6, IL-10, IL-8, IL-12p70, IFNγ, TNFα) were tested on Intellicyt iQue Screener and analyzed using ForeCyt analysis software. The cytokine (IFNα) was tested by standard ELISA. For 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.

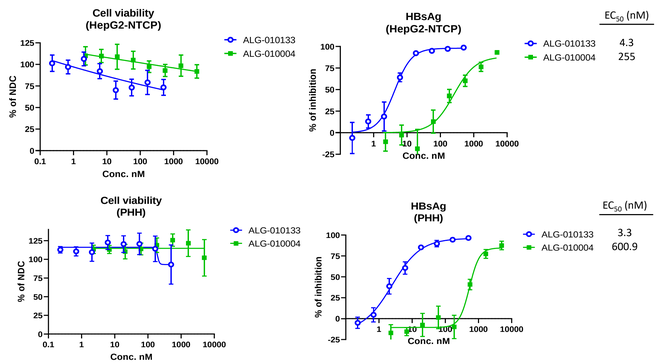
Results

STOPS Inhibit HBsAg Release in the HepG2.2.15 Cell Model



- HepG2.2.15 HBV Cell Model Optimized for Assaying STOPS
- Extensive STOPS SAR Explored
- ALG-010133: Approximately 30-fold More Potent in HepG2.2.15 HBsAg Release Assay than REP-2139

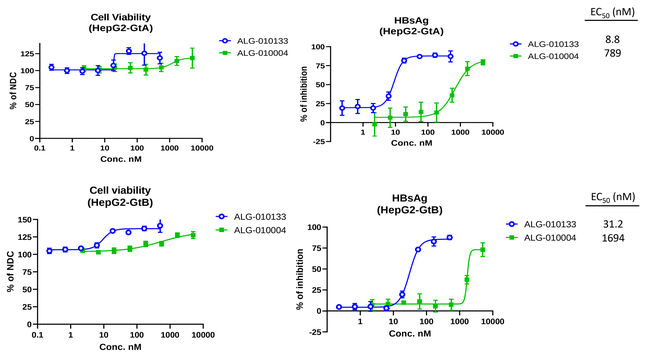
STOPS Inhibit HBsAg Release in Live HBV infection Models



- Live HBV infected HepG2-NTCP and PHH model optimized for assaying STOPS : HBsAg produced by HBV cccDNA
- STOPS Inhibitions of HBsAg release correlated well between HepG2.2.15 and HBV infection models
- ALG-010133 is approximately 60-fold more potent in HBV/HepG2-NTCP HBsAg release assay than the control
- ALG-010133 exhibits about 180-fold more potent activity in HBV/PHH HBsAg release assay than the control

Results

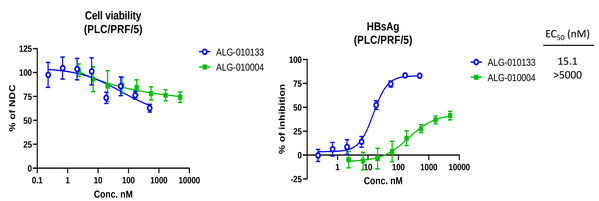
STOPS Demonstrate Pan-Genotypic Activity



Summary: Cross-Genotype Activity of ALG-010133 and ALG-010004

HBV Genotype	Compound	EC50 Value (nM) In Stable Cell Lines	Live HBV-Infected HepG2-NTCP	Live HBV-Infected PHH
A	ALG-010133	8.8 nM	–	–
	ALG-010004	789 nM	–	–
B	ALG-010133	31.2 nM	9.2 nM	–
	ALG-010004	1694 nM	71.9 nM	–
C	ALG-010133	–	0.7 nM	–
	ALG-010004	–	71.0 nM	–
D	ALG-010133	5.5 nM	4.3 nM	3.3 nM
	ALG-010004	149 nM	255 nM	600.9 nM

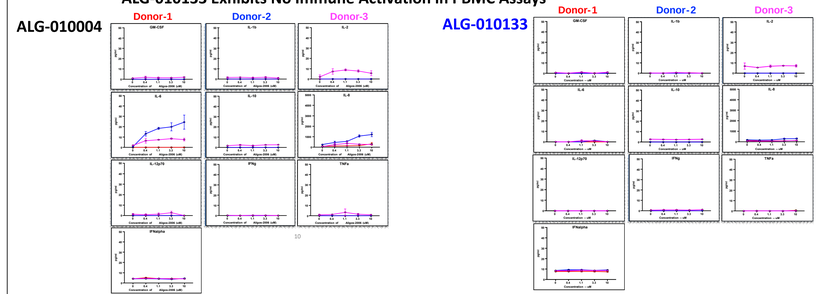
STOPS Inhibit HBsAg Release in PLC/PRF 5 Hepatoma Cells



- PLC/PRF5 is a cell model where HBsAg is produced from an integrated HBV genome
- ALG-010133 demonstrated more than 100-fold better potency than REP-2139

* ALG-010004: identical to REP-2139[3] (2'OMeA, 2'OMe-5-MeC)$_{20}$

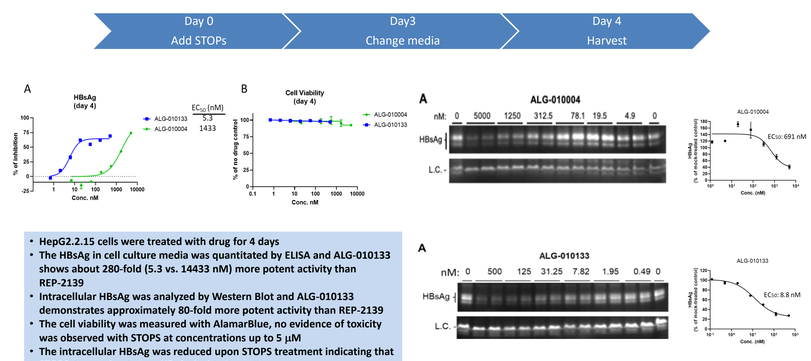
ALG-010133 Exhibits No Immune Activation in PBMC Assays



- To minimize injection site reactions as well as other adverse effects, selected STOPS™ show no immune activation in PBMC assays

Results

Intracellular HBsAg is Concurrently Reduced in HepG2.2.15 cells



- HepG2.2.15 cells were treated with drug for 4 days
- The HBsAg in cell culture media was quantitated by ELISA and ALG-010133 shows about 280-fold (5.3 vs. 14433 nM) more potent activity than REP-2139
- Intracellular HBsAg was analyzed by Western Blot and ALG-010133 demonstrates approximately 80-fold more potent activity than REP-2139
- The cell viability was measured with AlamarBlue, no evidence of toxicity was observed with STOPS at concentrations up to 5 μM
- The intracellular HBsAg was reduced upon STOPS treatment indicating that STOPS do not simply trap HBsAg inside the cells

ALG-010133 Combination Study Results

STOPS	Compound Class: ASO	NUC	CAM	Synergy Volume (μM²%)	Synergistic/ Antagonistic Interaction	Cytotoxicity
ALG-010133	ALG-020062*	–	–	291.6	Strong Synergistic interaction	No
ALG-010133	–	Entecavir	–	26.29	Additive to Minor Synergistic interaction	No
	–	Tenofovir	–	1.95	Additive interaction	No
ALG-010133	–	–	ALG-001075#	32.91	Additive to Minor Synergistic interaction	No

*ALG-020062 is an analog of the unconjugated form of ALG-020572, Aligos HBV ASO clinical candidate.
ALG-001075 is the active CAM component of pro-drug ALG-000184, Aligos HBV CAM clinical candidate.

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 in 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:
1. Al-Mahtab et. al. PLoS ONE (2016) , e 0156667
2. Bazinet et. al. Fri_210 EASL (2019) Vienna