Combination Drug Interactions of Hepatitis B Virus (HBV) S-antigen Transport-inhibiting Oligonucleotide Polymers In Vitro

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Abstract 431

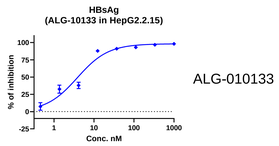
Background and Aims

Current standard of care for chronic hepatitis B (CHB) can effectively inhibit viral DNA replication but fails to reduce HBsAg that suppresses the human immune system. Previously, we have identified S-antigen Transport-inhibiting Oligonucleotide Polymers (STOPS) that share structural similarity with Nucleic Acid Polymers (NAPs) but contain several novel chemical features (AASLD 2020). STOPS can reduce HBsAg secretion by potentially inhibiting protein trafficking from the infected cell resulting in intracellular degradation of HBsAg. In this study, inhibition of HBV DNA or HBsAg secretion by STOPS was examined in pairwise or triple combinations with nucleos(t)ide analogs, core assembly modulators (CAMs), and HBV-specific antisense oligonucleotides (ASOs).

Methods

STOPS were synthesized on ABI 394 and Expedite 8909 synthesizers using standard phosphoramidite chemistry. In vitro combination studies were performed using the HepG2-derived HBV-producing stable cell line, HepG2.2.15. STOPS and ASO's were administered by transfection using RNAiMAX. Compounds were added to cells in a checkerboard fashion and inhibition of HBV replication measured by HBV DNA or HBsAg release assays 6 days after compound addition. Data were analyzed using the Bliss-Independence model using Pritchard's MacSynergy II.

ALG-010133 is a Potent Inhibitor of HBsAg Release



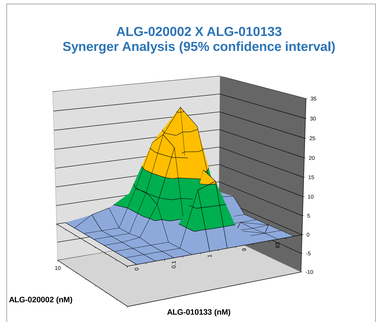
	HegG2-NTCP EC_{50} (nM)	HepG2.2.15 EC_{50} (nM)
REP 2139	462	482
ALG-010133	**3.2**	**3.9**

STOPS potently inhibit HBsAg release in vitro.

ALG-010133 reduced HBsAg secretion in a dose dependent fashion in HBV-infected HepG2-NTCP cells. Each concentration was performed in triplicate, and the plotted values represent the mean. ALG-010133 also reduced HBsAg in HepG2.2.15 cells (curve not shown) with a similar EC_{50} value (see table; EC_{50} values represent mean of 5 independent experiments). In both cell lines, ALG-010133 was ~100-fold more potent than the reference NAP.

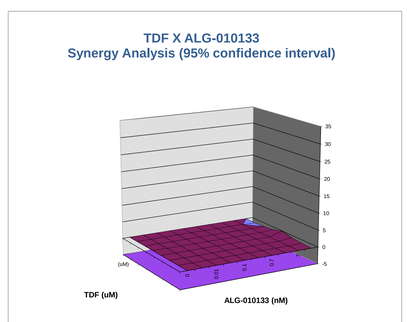
Combination Studies

A) Combination Studies with ASOs



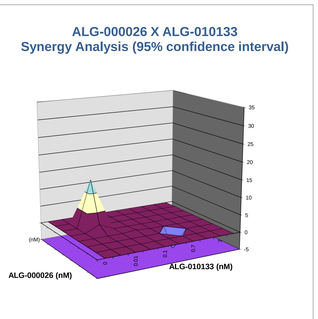
Synergy Volume 343 μM²%

B) Combination Studies with Nucleos(t)ide Analogs



Synergy Volume 1.95 μM²%

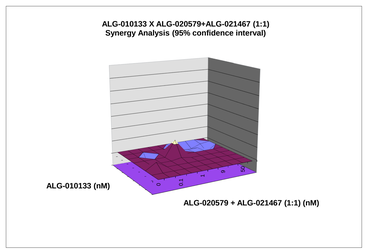
C) Combination Studies with Class II CAM Compounds

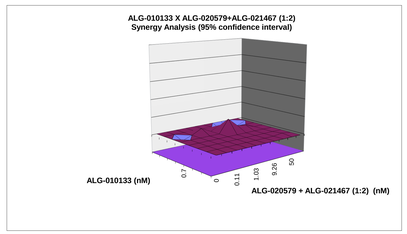


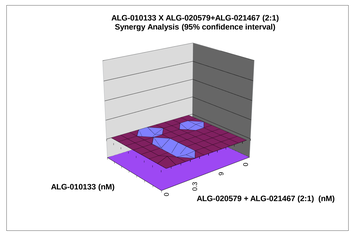
Synergy Volume 15.87 μM²%

STOPS				Synergy Volume (μM²%)	Synergistic / Antagonistic Interaction	Cytotoxicity
	ASO	NUC	CAM			
ALG-010133	ALG-020001	–	–	485.22	Strong Synergy	No
	ALG-020002	–	–	146.71	Strong Synergy	No
	ALG-020062	–	–	291.6	Strong Synergy	No
	–	Entecavir	–	26.29	Additive / Minor Synergy	No
	–	Tenofovir	–	1.95	Additive	No
	–	–	ALG-000026	15.87	Additive	No
	–	–	ALG-001024	1.26	Additive	No
	–	–	ALG-001075	32.91	Additive / Minor Synergy	No

D) Triple Combination Studies with ASOs







Compound	ASO S:X Ratio	Synergy Volume (μM²%)	Synergy Interaction	Cytotoxicity
	1:2	9.25	Additive	No
ALG-010133 (STOPS)	1:1	9.58	Additive	No
	2:1	0.99	Additive	No

Results

Pritchard's Model (MacSynergy II) Volume Descriptions

MacSynergy II Synergy/Antagonism Volumes Description @ 95% Confidence	
Volume (μM²%)	Volume Description
<25	Insignificant synergism/antagonism (additivity)
25-50	Minor synergism/antagonism
50-100	Significant synergism/antagonism – maybe important in vivo
>100	Strong synergism/antagonism – probably important in vivo
>1000	Probable errors

In HepG2.2.15 cells STOPS exhibit potent anti-HBsAg inhibitory activity with EC_{50} values in the low nanomolar range. ALG-010133 was tested in pairwise combinations with other inhibitors; HBV ASOs previous shown to reduce HBsAg release from HBV-infected cells, Class II CAMs, and nucleos(t)ide analogs. ALG-010133 demonstrated strong synergy when combined with the ASOs, ALG-020001, ALG-020002, and ALG-020062 in inhibiting HBsAg release in HepG2.2.15 cells.

When combined with the HBV nucleos(t)ide analogs entecavir or tenofovir, ALG-010133 demonstrated minor synergy or additivity in inhibiting HBsAg release, respectively, no antagonistic effects were observed.

When ALG-010133 was combined with the Class II CAMs, the interactive effect was additive with ALG-000026 and ALG-001024 and showed additive/minor synergistic effects with ALG-001075. None of the combinations demonstrated antagonistic effects or significant cytotoxicity.

Finally, when ALG-010133 was combined with combinations containing ASOs derived from ALG-020572 (S-trigger) and ALG-020576 (X-trigger) triple combinations demonstrated additive effects and no cytotoxicity.

Conclusions

Future functional cure for CHB will require a combination of compounds with different mechanisms of action. STOPS demonstrate an in vitro antiviral profile that suggests they may become an important component of a functional cure combination therapy. To this end, our STOPS compound is currently advancing towards combination clinical trials in CHB.

References

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Financial Disclosures

All authors are Aligos employees