Combination drug interactions of hepatitis B virus (HBV) small interfering RNA (siRNA) and antisense oligonucleotides (ASO) in vitro and in vivo

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ALIGOS THERAPEUTICS

Abstract #1257

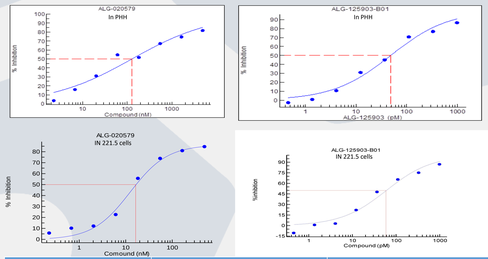
Background and Aims

Clinical studies have shown that siRNA's and ASO's targeting HBsAg knockdown are attractive therapeutic options for the treatment of chronic hepatitis B (CHB). We explored the combinations of an siRNA, ALG-125903 (unconjugated form of ALG-125755) and an ASO, ALG-020579 (unconjugated form of ALG-020572) in vitro in dual combinations with each other as well as with other anti-HBV agents such as nucleoside analogs (NA) and Capsid Assembly Modulators (CAM). We also explored the benefits of combining HBV siRNA (GalNAc conjugated ALG-125755) and ASO (GalNAc conjugated ALG-020572) constructs in the AAV-HBV model to maximize HBsAg reduction by utilizing their non-overlapping cellular pathways.

Methods

In vitro combination studies were performed using the HepG2.2.15 cell line. ALG-125903 and ALG-020579 were transfected using RNAiMAX into cells in a checkerboard fashion and HBsAg in the supernatant was measured by ELISA (enzyme-linked immunosorbent assay) 4 days post transfection. ALG-125903 or ALG-020579 combinations with CAM and NA were tested similarly with secreted HBV DNA as the endpoint. Drug-drug interaction data were analyzed by the Loewe additivity model and the Bliss independence drug interaction model. In vivo, ALG-125755 and ALG-020572 were administered subcutaneously (SC) in AAV-HBV mice as single agents as well as in combination. In the combination group, ALG-125755 was dosed as a single dose of 5 mg/kg on day 0 and ALG-020572 as repeat doses of 5 mg/kg on days 0, 7 and 14. Serial blood collections occurred every 5 days until day 60 for HBsAg ELISA and ALT assays.

ALG-020579 and ALG-125903 are Potent Inhibitors of HBsAg Release



	PHH EC50 (nM)	HepG2.2.15 EC_{50} (nM)
ALG-125903	0.03	0.05
ALG-020579	55.7	12.64

siRNA and ASO potently inhibit HBsAg release in vitro

ALG-125903 and ALG-020579 reduced HBsAg secretion in a dose dependent fashion in HBV infected PHH cells. Each concentration was performed in triplicate, and the plotted values represent the mean. Both also reduced HBsAg in PHH and HepG2.2.15 cells with no cytotoxicity.

COMBINATION STUDIES

Experimental design & analysis of synergy

Two experimental strategies were used to investigate antiviral synergy

Loewe Additivity Model:
The dose-effect curves for each drug was converted to median-effect plots

Triplicate data sets examined

Isobolograms graphically represent additive, synergistic and antagonistic drug effects. In this representation, an IC value of one drug is plotted on one axis and the corresponding IC value of a second drug is plotted on a second axis; the line connecting these two points represents the amount of each drug in a combination that would be required to reach the equivalent IC value provided their effects are additive. Synergistic action is indicated if lower doses of either agent can support an identical IC value. Ie., points fall below the line of additivity.

Drug Reduction Index (DRI) and *Combination Index (CI)* were calculated to quantify interdependence of antiviral effects

-DRI is a measure of how much the dose of each drug in a synergistic combination may be reduced at a given effect level cpmpared with the doses for each drug alone.
- A DRI is important in clinical situations when DRI>1, is beneficial indeed.

-CI takes into account the DRI of each agent: $CI = 1/(DRI)_{drug\ 1} + 1/(DRI)_{drug\ 2}$
-Synergy, CI = 1 - 0.3
-Strong synergy, CI = 0.1 - 0.3

Analysis performed in Calcusyn

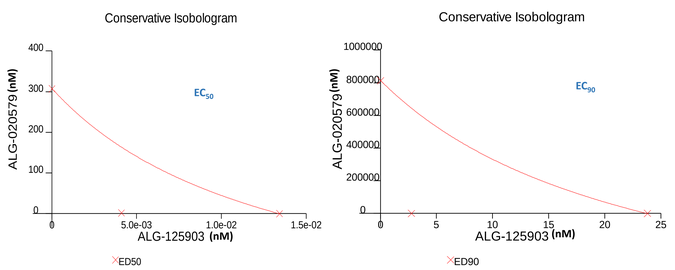
Bliss Independence Model
Nonparametric three-dimensional approach to quantify areas where observed effects are significantly greater (synergy) or less (antagonism) than those predicted from single-drug control data

Dose-response curves are generated using a checkerboard design in which drug ratios and concentrations were both varied

Analysis was performed in MacSynergy II

Triplicate data sets assessed at the 95% confidence level:
- *Minor synergy* = values >25 to <50 μM²
- *Moderate synergy* = values >50 to <100 μM² (log volumes >5 and <9)
- *Strong synergy* = values >100 μM² (log volume >9)

Loewe additivity modeling



	Combination Index (CI)		
Combination ratio (ALG-020579 : ALG-125903)	ED_{50}	ED_{75}	ED_{90}
170 : 1	0.37	0.13	0.04
500 : 1	0.31	0.19	0.11
5000 : 3	0.22	0.14	0.08

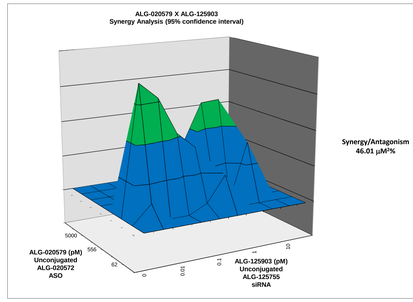
	Dose Reduction Index (DRI)					
	170 : 1		500 : 1		5000 : 3	
	ALG-020579	ALG-125903	ALG-020579	ALG-125903	ALG-020579	ALG-125903
EC_{50}	376.1	2.74	149.8	3.27	72.8	4.48
EC_{75}	1312.3	7.82	296.8	5.30	145.3	7.80
EC_{90}	4579.3	22.35	587.8	8.59	290.2	12.7

ALG-125903 and ALG-020579 show synergistic activity by Isobologram analysis and combination indices indicative of moderate to strong synergy

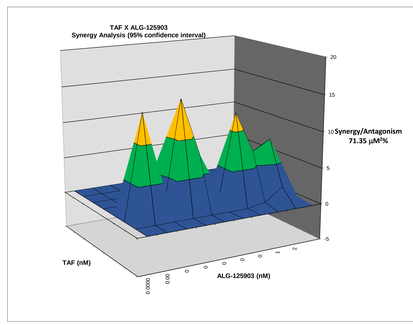
Drug reduction indices indicate doses of both can be reduced significantly and the same antiviral activity maintained when used in combination

Bliss independence modeling

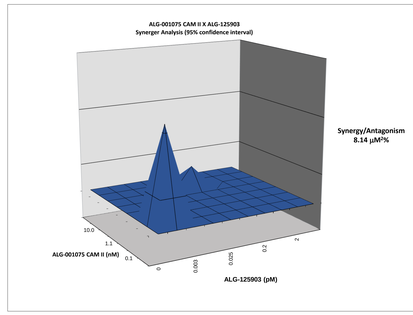
A) Combination Studies with ASOs



B) Combination Studies with Nucleos(t)ide Analogs



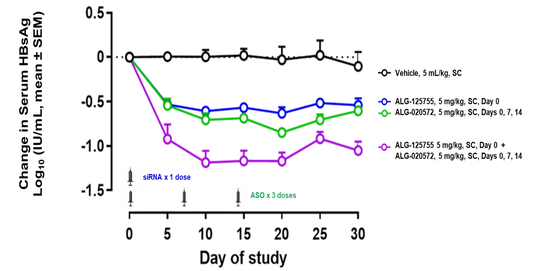
C) Combination Studies with Class II CAM Compounds



Compound		Synergy Volume (μM²%)	Synergy Interaction	Cytotoxicity
ALG-125903 (siRNA)	ALG-020579 (ASO)	46.01	Synergy	No
ALG-125903 (siRNA)	Tenofovir (NUC)	71.35	Synergy	No
ALG-125903 (siRNA)	ALG-001075 (CAM II)	8.14	Additive	No

Change in HBsAg in the Mouse AAV-HBV Model

ALG-020572 and ALG-125755 in vivo combination



The combination of our siRNA and ASO delivers rapid, potent suppression of HBsAg in vivo

Results

Combination of ALG-125903 and ALG-020579 in vitro exhibited minor synergy with a synergy volume of 46.01 μM²%. Combination of ALG-125755 and ALG-020572 in vivo demonstrated additive effects in HBsAg knockdown without change in serum ALT levels in mice. When tested in pairwise combinations with NA and CAM in vitro, HBV siRNA, ALG-125903 or ASO, ALG-020579 demonstrated significant synergy (synergy volume of >100 μM²%), synergy (25-100 μM²%) or additivity (0-25 μM2%), respectively. No antagonistic effects were observed.

Conclusions

The HBV siRNA, ALG-125755, in combination with the ASO, ALG-020572, demonstrated additive to minor synergy in vitro and in vivo. Further investigation of the strategy to combine HBV siRNA and ASO compounds in CHB clinical trials is warranted.

References

MacSynergy II software was kindly provided by Dr. M. Prichard (University of Michigan). CalcuSyn Version 2.0 by T-C Chou and P. Talalay, (**BIOSOFT,** Cambridge, United Kingdom).

Financial Disclosures

All authors are Aligos Therapeutics, Inc., employees