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TRIPLE COMBINATION OF ANTI-HEPATITIS B VIRUS DRUGS DEMONSTRATES SYNERGISTIC ACTIVITY IN VITRO

Hyunsoon Kang, Hua Tan, Jin Hong, Min Luo, Yuchun Nie, Rajendra Pandey, Saul Martinez Montero, Tilani De Costa, John Cortez, Vivek K. Rajwanshi, David B. Smith, Lawrence M. Blatt, Leonid N. Beigelman and Julian A. Symons
Aligos Therapeutics, Inc., 1 Corporate Drive, 2nd Floor, South San Francisco, CA 94080

Disclosure: All authors are current or former employees of Aligos Therapeutics, Inc.

ALIGOS THERAPEUTICS

Background

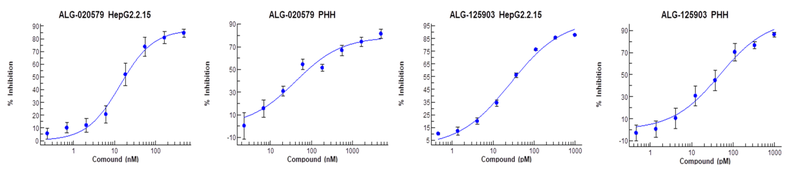
With high morbidity and mortality worldwide, there is a great need to develop effective therapies for chronic hepatitis B (CHB) patients. Previously, we have identified ALG-125903, a small interfering RNA (siRNA), ALG-020579, an antisense oligonucleotide (ASO) and ALG-010133, a S-antigen Transport-inhibiting Oligonucleotide Polymer (STOPS™) compound that can reduce HBsAg secretion. In this in vitro study, we examined the potential for combining these three compounds with nucleos(t)ide analogs (NA) or Capsid Assembly Modulators (CAM) in triple combinations.

Methods

In vitro triple combination studies were performed using the HepG2.2.15 cell line and HBV infected primary human hepatocytes (PHH). ALG-125903 and ALG-020579 or ALG-010133 were transfected using RNAiMAX into cells in a checkerboard fashion and third compound was either transfected at the same time or added to the cells post transfection. HBsAg in the supernatant was measured by ELISA (enzyme-linked immunosorbent assay) 5 days post transfection for the combination with IFN or STOPS™ (ALG-010133). ALG-125903 or ALG-020579 combinations with CAM II (ALG-000111) and NAs, Tenofovir (TDF) or Entecavir (ETV), were tested similarly with qPCR (quantitative polymerase chain reaction) determination of secreted HBV DNA as the endpoint. Data were analysed using Calcsyn software for Loewe Additivity Model or MacSynergy II software for Bliss Independence Model.

Experiments

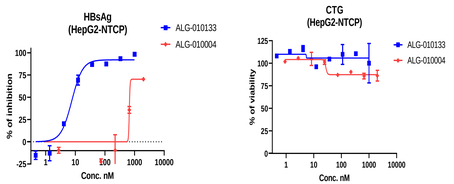
ALG-125903 and ALG-020579 are Potent Inhibitor of HBsAg Release



	PHH		HepG2.2.15	
N=3	EC_{50} (nM)	CC_{50} (nM)	EC_{50} (nM)	CC_{50} (nM)
ALG-125903	0.03	>5000	0.05	>500
ALG-020579	55.7	>5000	12.64	>500

siRNA and ASO potently inhibit HBsAg release in vitro
ALG-125903 and ALG-020579 reduced HBsAg secretion in a dose dependent fashion in HepG2.2.15 Cell line as well as HBV infected PHH. The plotted values represent the average of three experiments.

STOPS™ inhibit HBsAg Release in Live HBV / HepG2-NTCP Cells



	Live HBV / HepG2-NTCP		HepG2.2.15	
	EC_{50} (nM)	CC_{50} (nM)	EC_{50} (nM)	CC_{50} (nM)
ALG-010004*	665	>2000	442	>2000
ALG-010133	4.9	>2000	3.5	>2000

*ALG-010004 has an identical oligonucleotide sequence as REP 2139[1]
N=3

- **Live HBV HepG2-NTCP model optimized for assaying STOPS™: HBsAg produced by HBV cccDNA**
- **STOPS™ inhibition of HBsAg release correlated well between HepG2.2.15 and HBV/HepG2-NTCP**
- **ALG-010133: >100X more potent in HBV/HepG2-NTCP HBsAg release assay than (2'OMeA, 2'OMe-5-MeC)20**

Experiments

Experimental design & analysis of synergy

Two experimental strategies were used to investigate antiviral synergy

Loewe Additivity Model[3]:
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. I.e., 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 compared 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[2]

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)**

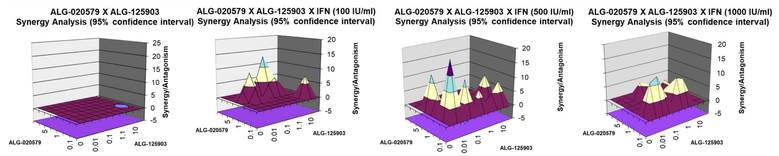
Triple Combination Showed Synergistic Activity Using The Loewe Additivity Model

Combination Index (CI)			
Combination ratio	ED_{50}	ED_{75}	ED_{90}
ALG-020579 X ALG-125903 (30:50)	0.30	0.29	0.31
ALG-020579 X IFN (10000 : 5)	0.99	0.13	0.06
ALG-125903 : IFN (10000 : 3)	0.33	0.04	0.005
IFN : ALG-125903 : ALG-020579 (10000 : 100 : 50)	0.54	0.20	0.24

Dose Reduction Index (DRI)			
Combination ratio (10000 : 100 : 50)	IFN	ALG-125903	ALG-020579
ED_{50}	2.46	11.23	21.05
ED_{75}	24.61	14.87	10.65
ED_{90}	246.69	19.68	5.39

- **Combination index indicates combination between siRNA X ASO, ASO X IFN, siRNA X IFN and triple combination synergistic relationship**

ALG-020579 X ALG-125903 X IFN Showed Moderate Synergy in Inhibiting HBsAg Release in HepG2.2.15

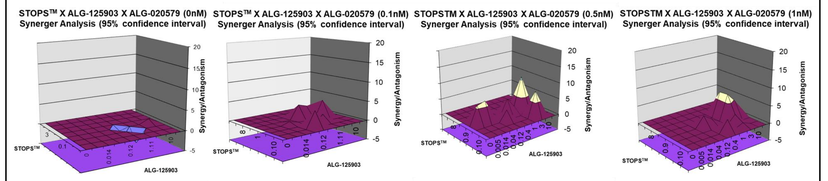


	IFN (IU/ml)			
ALG-020579 X ALG-125903	0	100	500	1000
Synergy Volume (uM²%) (95% Confidence Level)	34.63	59.95	92.6	80.28

- **ALG-020579 combined with ALG-125903 showed minor synergy in inhibiting HBsAg release in HepG2.2.15 cell line**
- **Addition of IFN significantly increased synergy volume indicating enhanced synergistic interaction of ALG-020579 and ALG-125903 when combined with IFN**

Experiments

ALG-020579 X ALG-125903 X STOPS™ Showed Additive to Minor Synergy in Inhibiting HBsAg Release



Synergy Volume (uM²) (95% Confidence Level)	ALG-020579 (nM)			
STOPS™ X ALG-125903	0	0.1	0.5	1
HepG2.2.15	1.13	23.68	34.23	29.22
PHH	2.4	37.31	7.66	0

- **STOPS™ combined with ALG-125903 showed additive effect in inhibiting HBsAg release using HepG2.2.15 cell line and HBV infected PHH**
- **Addition of ASO increased synergy volume in HepG2.2.15, indicating that ALG-020579 can enhance the combination effect of STOPS™ and ALG-125903 to minor synergistic interaction**

In-Vitro Triple Combination Showed Additive to Moderate Synergy

	ALG-020579 X ALG-125903 Combination Index				
3rd Compound Doses	IFN*	Tenofovir**	Entecavir***	CAM II****	STOPS™*****
0	Minor Synergy	Minor Synergy	Minor Synergy	Minor Synergy	Additive
1	Moderate Synergy	Additive	Minor Synergy	Minor Synergy	Additive
2	Moderate Synergy	Minor Synergy	Minor Synergy	Minor Synergy	Minor Synergy
3	Moderate Synergy	Additive	Additive	Minor Synergy	Minor Synergy

*Doses for IFN 1=100, 2=500, 3=1,000 IU/ml, **Tenofovir 1= 0.2, 2=0.7, 3=2 uM, ***Entecavir 1=5, 2=10, 3=25 (nM), CAM II**** 1=0.1, 2=0.3, 3=1 (nM), and *****STOPS™ 1=0.1, 2=0.5, 3=1 (nM) (ALG-020579 was used as 3rd compound). Synergy Volume was calculated with HBsAg for IFN and STOPS™. For TDF, ETV and CAM II, Synergy Volumes were determined using extracellular DNA.

Results

Triple combinations of ALG-125903 and STOPS™ (ALG-010133) with ALG-020579 showed additive activity and demonstrated a dose dependent response with respect to ALG-020579. By Bliss analysis, this combination exhibited a synergy volume of 1.13 μM²% increasing to 23.68 μM²% to 34.23 μM²%. Effect of ALG-125903 in combination with ALG-020579 were enhanced when IFN was added to the combination with synergy volume increase in a dose dependent manner. When tested in pairwise combinations with a NA or CAM, the compounds demonstrated additive to synergistic interactions on inhibition of HBV DNA. No antagonistic effects were observed.

Conclusions

ASO ALG-020579 and siRNA ALG-125903 are potent inhibitors of HBsAg release. Combination of ALG-020579 and ALG-125903 showed minor synergistic interaction on HBsAg release. When IFN or STOPS™ were added to the ASO and siRNA combination, the effect was enhanced indicating the possibility of lowering doses needed to treat HBV when combined with other HBV therapies.

References

1. A. Vaillant, Antiviral Research 133 (2016) 32.
2. MacSynergy II software was kindly provided by Dr. M. 2. Prichard (University of Michigan).
3. CalcuSyn Version 2.0 by T-C Chou and P. Talalay, (**BIOSOFT,** Cambridge, United Kingdom).