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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Background and Aims
Clinical studies have shown that siRNAs and ASOs 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 Modulation (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-125903 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

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
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