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 potently 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 Expedit 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 RNAmax. 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.

Results
Pritchard’s Model (MacSynergy II) Volume Descriptions

<table>
<thead>
<tr>
<th>MacSynergy II Synergy/Antagonism Volumes Description @ 95% Confidence</th>
<th>Volume Description</th>
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</thead>
<tbody>
<tr>
<td>&lt; 25</td>
<td>maybe important in vivo</td>
</tr>
<tr>
<td>25–100</td>
<td>– maybe important in vivo</td>
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<tr>
<td>&gt; 100</td>
<td>– probably important in vivo</td>
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<tr>
<td>&gt; 1000</td>
<td>Probable errors</td>
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In HepG2.2.15 cells STOPS exhibit potent anti-HBsAg inhibitory activity with EC50 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-0200572 (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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