Structural requirements for S-antigen transport-inhibiting oligonucleotide polymer inhibition of hepatitis B surface antigen secretion

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ALIGOS THERAPEUTICS

Abstract SAT449

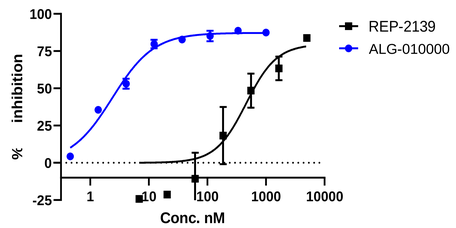
Background and Aims

A functional cure for chronic hepatitis B is unavailable for all patients, as most current therapeutics lack mechanisms for viral surface antigen (HBsAg) reduction. S-antigen Transport-inhibiting Oligonucleotide Polymers (STOPS™) reduce HBsAg *in vitro* and are structurally similar to nucleic acid polymers (NAPs). Novel chemical properties have imparted 20 to 100-fold improvements in potency over clinical stage NAPs. To identify the mechanism for enhanced potency, we investigated the structural features necessary for STOPs antiviral activity via modifications of sequence, length and chemistry.

Methods

To assess STOPS antiviral activity, HBsAg levels were measured in treated and untreated HBV-infected cells. Briefly, STOPS were transfected into HepG2.2.15 and HBV-infected HepG2-NTCP cells. HBsAg levels and cytotoxicity were measured 6 days post transfection via ELISA and CellTiter Glo, respectively.

Chemical modifications improve potency of STOPS 100x vs. NAPs

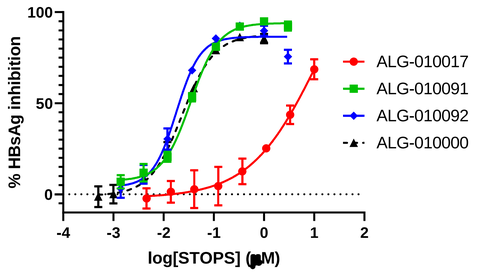


	HegG2-NTCP EC_{50} (nM)	HepG2.2.15 EC_{50} (nM)
REP 2139	462	482
ALG-010000	**5.3**	**4.6**

Figure 1. ALG-010000 is a potent inhibitor of HBsAg in vitro. *ALG-010000 reduced HBsAg 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-010000 also reduced HBsAg in HepG2.2.15 cells (curve not shown) with a similar EC_{50} value (see table; EC_{50} values represent mean from 5 independent experiments). In both cell lines, ALG-010000 was 100-fold more potent than the reference NAP.*

Probing the effect of size and sequence on STOPS activity

Optimal STOPS activity requires > 34 nucleotides

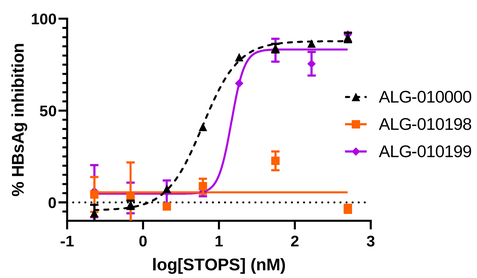


	Length	Percent activity of 40mer ALG-010000 (%)
ALG-010017	17	2
ALG-010091	34	67
ALG-010092	37	129
ALG-010000	40	100

Figure 2. The antiviral activity of STOPS is size dependent. *Activity is maintained at >34 nucleotides, as demonstrated with the comparable potency of the 37mer ALG-010092 to the 40mer ALG-010000. ALG-010091, which is 34 nucleotides in length, retains 67% activity of ALG-010000. Potency dramatically reduced at lengths <30 nucleotides, as demonstrated by the 98% reduction in activity of ALG-010017, which is 17 nucleotides in length. Values plotted are the mean of duplicate measurements.*

Sequence-dependent structure of STOPS impacts potency

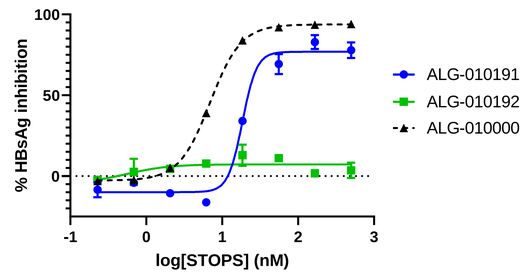
A)



	Sequence	EC_{50} (nM)
ALG-010000	Reference $(AC)_{20}$	6.1**
ALG-010198	$(AG)_{20}$	>500
ALG-010199	$(CA)_{20}$	14.7
ALG-010200*	$(AA)_{20}$	>500
ALG-010202*	$(CC)_{20}$	>500

* Data points not plotted in graph
** Value from plotted experiment and run on the same plate

B)

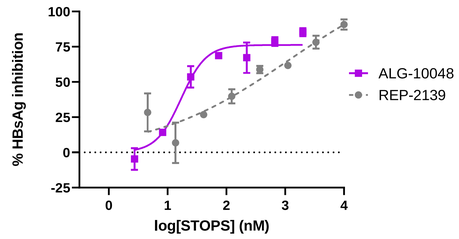


	Sequence	EC_{50} (nM)
ALG-010000	Reference $(AC)_{20}$	6.9**
ALG-010191	10 x A stretch (5′ and 3′ end)	18.5
ALG-010192	10 x C stretch (5′ and 3′ end)	>500

** Value from plotted experiment and run on the same plate

Figure 3. STOPS antiviral activity is dependent on A and C content. A) *Non-AC repeated bases, polyA and polyC sequences with the same chemistry as ALG-010000 were inactive, as demonstrated by the lack of HBsAg reduction in HepG2.2.15 cells by ALG-010198, ALG-010200 and ALG-010202, respectively. This contrasts with the reported activity for NAPs.[1] The sequence induced changes in STOPS secondary structure is hypothesized to cause losses in potency. Values plotted are the mean of duplicate.* ***B)*** *PolyA stretches replacing 2–5 dinucleotide repeats at the flanks of the 40mer oligonucleotide were tolerated while polyC stretches were detrimental. ALG-010191, with a 10 A stretch at both the 5′ and 3′ end of the STOPS, reduced HBsAg in HepG2.2.15 with an EC_{50} value of 18.5 nM (values plotted are the mean of duplicate). In contrast, ALG-010193, which instead replaced the ten 5′ and 3′ nucleotides with C, was inactive and did not reduce HBsAg even at the highest concentration.*

Backbone chemistry modulates antiviral activity



	Modification	HepG2.2.15 EC_{50} (nM)
REP 2139	$(AC)_{20}$ [2′-OMe-A, 2′-OMe-5-MeC, all ps]	482
ALG-010048	20R isomers	**19.8**

Figure 4. Incorporation of stereospecific phosphorothioate bonds improves potency in vitro. *When 20 R isomers are incorporated into a phosporothioated 40mer with 2′OMe-A and –C repeats, the EC_{50} value for HBsAg reduction in HepG2.2.15 is improved 24-fold over the non-modified, racemic mixture control, REP 2139. This supports the importance of structure on the activity of STOPS and related molecules.*

Conclusions

We have demonstrated that STOPS potency is size and sequence dependent, requiring a minimum of 34 nt and a minimal AC dinucleotide composition of 50%. Chemistries that improve antiviral activity have also been identified. Collectively, these defined structural elements provide a framework for STOPs design and are important for their advancement.

References

1) **Guillot C et al.** Inhibition of hepatitis B viral entry by nucleic acid polymers in HepaRG cells and primary human hepatocytes. *PLOS One 2017; 12(6): e0179697*.

Financial Disclosures

All authors are Aligos employees