Background and Aims

A functional cure for chronic hepatitis B is unavailable for all patients; 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

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 EC50 value (see table; EC50 values represent mean of 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

Figure 2. The antiviral activity of STOPS is size dependent. Activity is maintained at >34 nucleotides, as demonstrated with the comparable potency of the 17mer ALG-010092 to the 40mer ALG-010000. ALG-010092, 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

Figure 3. STOPS antiviral activity is dependent on A and C content. A) Non-AC repeat 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. 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 EC50 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.

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


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All authors are Aligos employees.