

# Improved Liver Concentration and Activity of S-antigen Transport-Inhibiting Oligonucleotide Polymers

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## Background

Therapeutics aiming to cure chronic hepatitis B via hepatitis B virus (HBV) surface antigen (HBsAg) reduction require delivery to infected hepatocytes to impart efficacy. S-antigen Transport-inhibiting Oligonucleotide Polymers (STOPS™) reduce HBsAg in vitro and exhibit 20-100-fold enhanced potency over clinical stage, structurally similar nucleic acid polymers (NAPs). Challenges for nucleic acid-based therapies include tissue targeting, however, N-acetylgalactosamine (GalNAc) mediated delivery via the hepatocyte enriched asialoglycoprotein receptor has been demonstrated to target oligonucleotides to the hepatocytes. Here we describe the tissue levels of GalNAc-conjugated STOPS in mice and non-human primates (NHP), and their antiviral activity in an in vitro 3D liver system without transfection.

## Methods

To assess STOPS biodistribution in mice and NHPs, selected tissues were harvested 24 or 48 hours post subcutaneous dosing of STOPS and concentrations were analyzed using LC-MS/MS. Fluorophore-labeled STOPS were also dosed in mice for whole-body in-life imaging and terminal organ imaging. HBV-infected 3D liver chips were used to evaluate the antiviral activity of the STOPS, which were added every 3 days for 30 days. HBsAg and human albumin levels were measured via ELISA. Alanine aminotransferase levels were measured using an enzymatic activity assay.

## STOPS Potently Inhibit HBsAg Secretion In Vitro

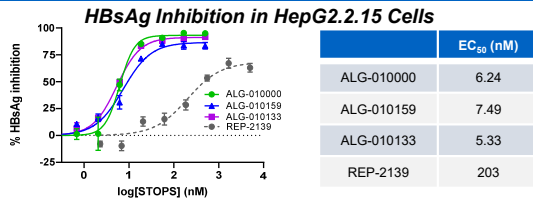


Figure 1. ALG-010000 and ALG-010159 demonstrate potent in vitro EC<sub>50</sub> values for HBsAg inhibition as compared to the clinical molecule ALG-010133 as well as REP2139. STOPS are transfected into HepG2.2.15 cells, and supernatants are evaluated by ELISA for HBsAg quantification.



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## Optimizing GalNAc Conjugation to STOPS for Enhanced Liver Delivery

### GalNAc-Conjugation Using Linkers Improves STOPS Liver Concentration by 3-Fold in Mice

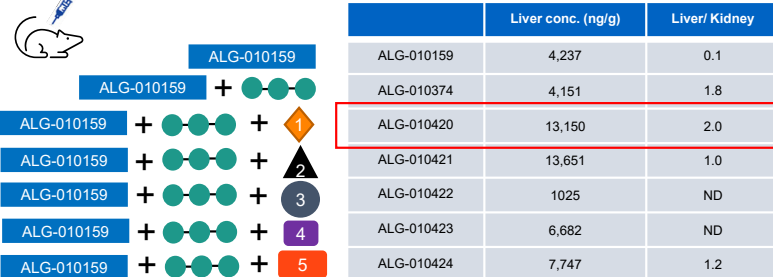


Figure 2. Liver concentration and liver-to-kidney ratio of STOPS improves in mice dosed with ALG-010159 with GalNAc-linker conjugation, but not with direct conjugation. STOPS were dosed SC at single dose 5 mg/kg in mice (n=4/group), and liver concentrations of ALG-010159 are reported at a 24 hr. post-dose timepoint; ND =not determined. Green circles in schematic on left indicate Aligos GalNAc moiety, and various shapes indicate linkers differing in stability and size. ALG-010420 (boxed) demonstrates improved liver concentration of 3x and liver/kidney ratio of 20x over ALG-010159 (unconjugated) as compared to STOPS with other linkers.

### Biodistribution of Fluorophore-Labeled STOPS in Live Mice Confirm Liver Targeting

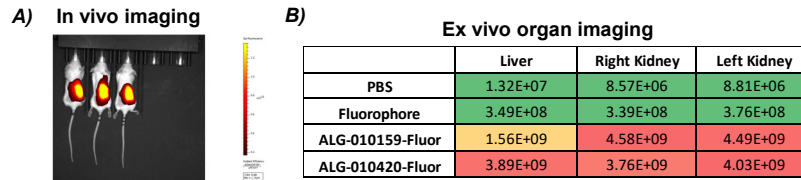


Figure 3. Mean radiant fluorescence of organs from mice dosed with optimized GalNAc-conjugated STOPS demonstrates improved liver targeting. STOPS (or fluorophore alone) were dosed SC at 5 mg/kg (corrected for added fluorophore MW) in mice (n=3/group), and whole-body IVIS images were taken over 48 hr., after which mice were sacrificed. The organs were imaged at the terminal timepoint (48 hr.). A) ALG-010159 dosed mice at 1 hr. post-dose. B) Mean radiant efficiency quantification of fluorescence at 48 hr. post-dose for the liver and kidney demonstrate improved liver concentrations of ALG-010159 when conjugated with an GalNAc-linker ("ALG-010420-Fluor").

## STOPS Liver Targeting in NHP

Conjugation	Mouse liver conc. (ng/g)	NHP liver conc. (ng/g)
ALG-010159	4,237	44,400
ALG-010420	GalNAc-linker ↑	75,350
ALG-010133	4,571	14,200
ALG-010474	GalNAc-linker ↑	33,350

Figure 4. Liver levels of the clinical molecule, ALG-010133 also improves with optimal GalNAc-linker conjugation. Like ALG-010159, ALG-010133 liver concentrations are improved 3-fold in mice and 2-fold in NHP. For both species, STOPS were dosed SC at 5 mg/kg (mice: n=4/group, NHP: n=2/group), and liver concentrations are reported at 24 hr. and 48 hr. post-dose for mice and NHP, respectively.

## GalNAc-STOPS Reduce HBsAg in 3D Liver-Chip

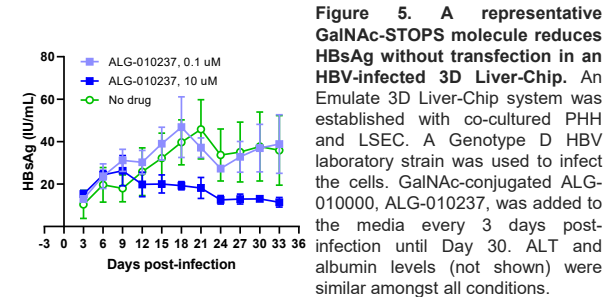


Figure 5. A representative GalNAc-STOPS molecule reduces HBsAg without transfection in an HBV-infected 3D Liver-Chip. An Emulate 3D Liver-Chip system was established with co-cultured PHH and LSEC. A Genotype D HBV laboratory strain was used to infect the cells. GalNAc-conjugated ALG-010000, ALG-010237, was added to the media every 3 days post-infection until Day 30. ALT and albumin levels (not shown) were similar amongst all conditions.

## Conclusions

GalNAc conjugation with an optimized linker design significantly increased STOPS concentrations in mouse and NHP livers. Furthermore, hepatocyte delivery correlated with in vitro antiviral activity in the 3D liver system. Improved liver concentrations and activity of GalNAc-conjugated STOPS indicates a potential to significantly reduce the effective antiviral dose for patients.

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