Background and Aims
Reducing hepatitis B virus (HBV) S antigen (HBsAg) is key to achieving functional cure in chronic hepatitis B (CHB) patients. Antisense oligonucleotides (ASOs) are effective in reducing HBsAg in animal models and CHB patients. However, hepatotoxicity is a major side effect of ASO administration that can be exacerbated with higher affinity Locked Nucleic Acid (LNA) modified ASOs. Next generation Bridged Nucleic Acid (BNA) and nucleobase modified manifolds can reduce hepatotoxicity while maintaining efficacy. We have therefore applied these chemistries in our LNA-containing HBV targeting ASOs.

Methods
ASOs with LNA and BNA chemistries were synthesized on ABI 394 and Expedite 8909 synthesizers using standard phosphoramidite chemistry. In vitro screening of LNA ASOs was carried out in HepG2/2.15 cells using a HBsAg release assay. Potent LNA-containing ASOs were chosen for conjugation with our proprietary N-Acetylgalactosamine-4 (GalNAc)-containing ASO monomers. BNA wing and nucleobase modifications were applied using an in-house algorithm and we compared these constructs to the all-LNA ASO parents in the adenovirus-associated virus (AAV)-HBV mouse model.

Wing Modification with Luxna 3rd Gen BNA Improved Potency and Reduced Liver Toxicity

Figure 2. A single Scp BNA substitution in the wing of all LNA ASOs increased in vivo potency and reduced liver toxicity. ALG-020572 is identical to ALG-020089 except that the 2nd position in the 3rd wing contains [5mScp C instead of LNA C]. B) In the AAV-HBV mouse model, both oligonucleotides were dosed subcutaneously 3x10mg/kg weekly. Serial blood collections were taken every 5 days and HBsAg ELSA was used to measure serum levels. With 3rd Gen BNA chemistry, the HBsAg nadir was improved 0.5 log, (h)(m). C) ALT measurement in the same samples indicated that maximum ALT was reduced 3-fold with 3rd Gen BNA.

Gap Modification with Luxna Nucleobase Reduced Liver Toxicity While Maintaining Potency

Figure 3. A single (2J[t] substitution in the DNA Gap of ASO eliminated liver tox while maintaining potency. ALG-020900 is all LNA HBV ASO with all DNA Gap. A) ALG-020929 is identical to ALG-020900 except that the 3rd position of the gap contains (2J[t] instead of DNA T). B) In AAV-HBV mouse model, both oligos were dosed subcutaneously 3x10mg/kg, weekly. Serial blood collections were made every 5 days and HBsAg ELSA was carried out in the serum. With nucleobase modification, in vivo potency was maintained. C) When ALT assay was conducted in the same serum the nucleobase gap modification eliminated liver tox with maximum ALT reduced 20 times.

Conclusions
A) Applying Luxna chemistries, including 3rd generation BNA wing and nucleobase gap modifications can increase therapeutic index as demonstrated by in vivo studies in the AAV-HBV model. B) ALG-020572 and ALG-020576 are HBV ASO development candidates incorporating Luxna chemistries that target the HBV S and X regions respectively. Both compounds demonstrated enhanced in vivo potency when compared to the Ph2 clinical compound, GSK836. C) Combination of ALG-020572 (S) and ALG-020576 (X) may have multiple advantages. Results from in vitro and in vivo combination studies are pending.

References
1) Setoguchi K. et al. Molecular Therapy: Nucleic Acids Vol. 9 December 2017
2) Kobori T. et al. Poster O66 OT5 2019

Financial Disclosures
All authors are Aligos Therapeutics employees.