BEST-IN-CLASS ANTISENSE OLIGONUCLEOTIDES AGAINST HEPATITIS B VIRUS: NEXT GENERATION BRIDGED NUCLEIC ACID CHEMISTRIES SIGNIFICANTLY INCREASE IN VIVO EFFICACY AND REDUCE HEPATOTOXICITY IN MICE

AASLD The Liver Meeting® Nov. 12-15, 2021

Publication Number: 820

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ALIGOS THERAPEUTICS

Background

Reducing HBsAg is key to achieve functional cure in chronic hepatitis B (CHB) patients. Antisense oligonucleotides (ASOs) are effective in reducing HBsAg in animal models, and patients with CHB. Hepatotoxicity is a major side effect of ASOs and is exacerbated with high affinity more active Locked-Nucleic Acid (LNA) modified ASOs. Next generation Bridged Nucleic Acid (BNA) monomers can reduce hepatotoxicity while maintaining efficacy. We have therefore utilized these BNA chemistries in our LNA-containing hepatitis B virus (HBV) targeting ASOs.

Methods

In vitro screening of LNA ASOs was carried out in HepG2.2.15 cells using an HBsAg release assay. A potent LNA-containing ASO was chosen for N-acetylgalactosamine (GalNAc) conjugation and dosed twice weekly in the adeno-associated virus (AAV)-HBV mouse model and exploratory mouse toxicity studies. BNA wing and nucleobase gap modifications were applied and compared to the parent all-LNA ASO.

ASOs for Treatment of CHB: 1 X-Region Sequence Identified

ASO #	Modifications	GalNAc
ALG-020039	None; parent ASO sequence	No
ALG-021544	Only LNA and DNA	No
ALG-020459	Only LNA and DNA	Yes; proprietary
ALG-021467	LNA + DNA + proprietary BNA monomers	No
ALG-021548	LNA + DNA + proprietary BNA monomers	Yes; non-proprietary
ALG-020576	LNA + DNA + proprietary BNA monomers	Yes; proprietary

HBV Genotypic Coverage of ALG-020039 Sequence

Genotype	A	B	C	D	E	F	G	H	I	J
% Homology*	99%	100%	99%	100%	96%	100%	99%	100%	100%	97%

* Including perfect match and 1 mismatch

Bioinformatic Analysis of Potential Off-Targets for ALG-020039

ASO	Human Spliced			Human Un-spliced			Cyno Spliced			Cyno Un-spliced			Mouse Spliced			Mouse Un-spliced		
	0 M	1 M	2 M	0 M	1 M	2 M	0 M	1 M	2 M	0 M	1 M	2 M	0 M	1 M	2 M	0 M	1 M	2 M
ALG-020039	0	0	3	0	0	5	0	0	6	0	0	8	0	0	2	0	0	3

M = mismatch

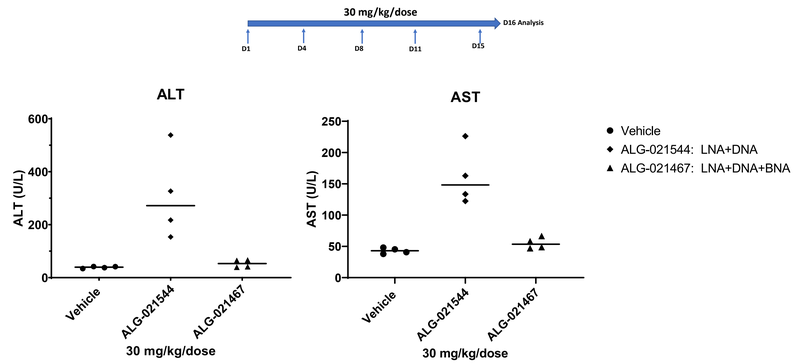
- High genotypic coverage for Hepatitis B virus (HBV)
- Sequence highly specific for HBV; low potential for off-target effects

Potency and In Vitro Cytotoxicity of ASOs with Same Sequence

ASO	IC_{50} (nM)	CC_{50} (nM)
ALG-021544	0.14	23.2
ALG-021467	0.28	>100
ALG-020576	2.24	>100

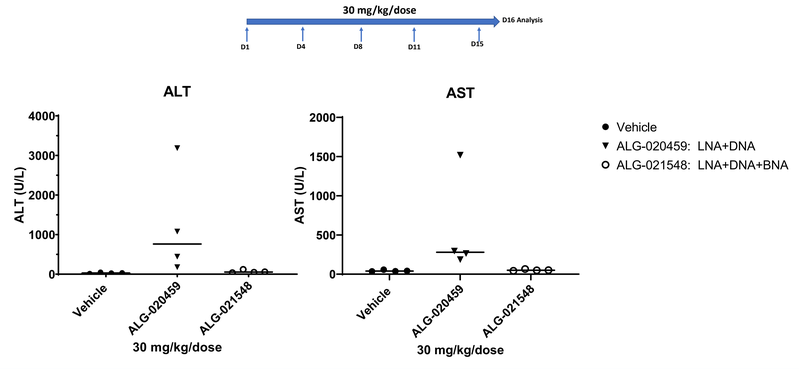
- Potency of unconjugated ASO maintained and cytotoxicity reduced with chemical modifications
- In vitro potency of GalNAc conjugated ASO (ALG-020576) was lower, but effectively delivers the more potent unconjugated ASO (ALG-021467) to the target hepatocytes

Toxicity of Unconjugated Sequence ± Chemical Modifications in Male Mice



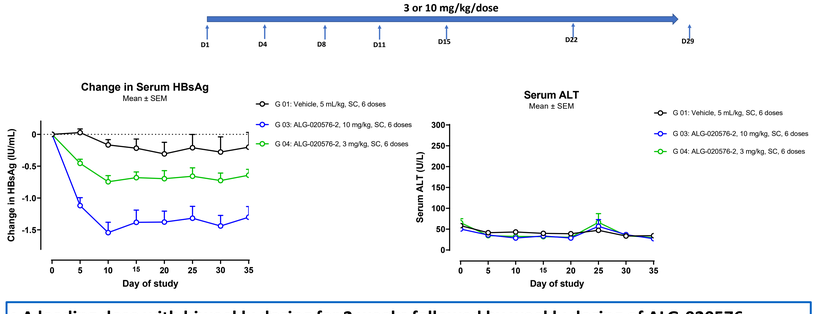
- Chemical modifications significantly reduce liver toxicity in mice without GalNAc conjugation

Toxicity of GalNAc-Conjugated Sequence ± Modifications in Male Mice



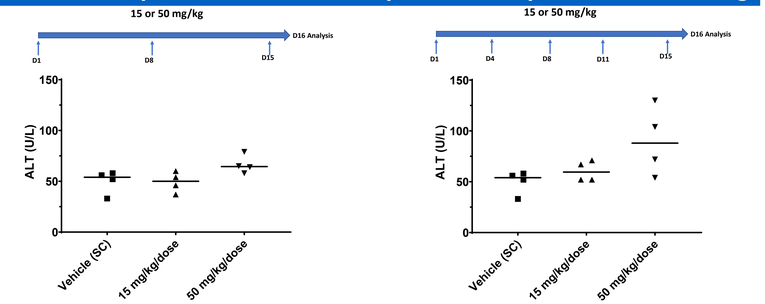
- Chemical modifications significantly reduce liver toxicity in mice with GalNAc conjugation

Dose-Dependent HBsAg with SC Loading Dose of ALG-020576

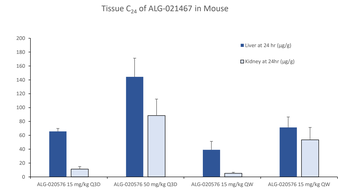


- A loading dose with biweekly dosing for 2 weeks followed by weekly dosing of ALG-020576 showed maximal efficacy in mouse model at 10 mg/kg/dose with no increase of ALT

No Liver Toxicity Observed with Weekly or Bi-Weekly ALG-020576 Dosing



High Liver Levels Observed in Mice with Repeat ALG-020576 Dosing



SC Dose Group (mg/kg/dose)	Liver C_{24} ± SD (µg/g)	Kidney C_{24} ± SD (µg/g)	Liver to Kidney Ratio
Q3D 5x15	66.7 ± 4.1	11.4 ± 3.6	6.3
Q3D 5x 50	144 ± 27.0	88.4 ± 23.9	1.8
QW 3x15	39.3 ± 12.2	5.3 ± 1.5	8.1
QW 3x50	71.3 ± 15.2	53.4 ± 18.0	1.4

ALG-020576 Demonstrated a Favorable Efficacy and Safety Profile

- ALG-020576 has >95% homology across all HBV genotypes and low off-target potential
- Proprietary BNA chemical modifications of the same sequence showed that the vitro potency was maintained while improving viability compared to the LNA-and DNA-containing parent
- In the AAV-HBV mouse model, ALG-020576 demonstrated a dose-responsive reduction in HBsAg and could achieve maximal HBsAg knockdown > 1.5 $\log_{10}$ IU/mL with no increase of ALT
- In mouse exploratory toxicity studies, LNA- and DNA-containing parent at 30 mg/kg/dose (with or without GalNAc conjugation) demonstrated elevations of ALT/AST, along with microscopic changes consisting of hepatocellular apoptosis/necrosis indicative of liver toxicity
- When BNA chemical modifications were made to the LNA- and DNA-containing parent (with or without GalNAc conjugation), no increases in ALT or AST were observed, and no adverse microscopic changes were noted in the liver at the same 30 mg/kg/dose level
- ALG-020576 was further profiled and demonstrated no liver toxicity up to 50 mg/kg/dose when dosed either weekly (3 doses) or bi-weekly (5 doses) for 2 weeks
- High liver concentrations of the potent unconjugated parent ALG-021467 were observed when dosed with ALG-020576
- Conjugation of the proprietary GalNAc also demonstrated favorable liver partitioning over kidney, particularly with weekly dosing

Conclusions

We demonstrated that applying next generation BNA and nucleobase chemistries in LNA ASO gapmers can significantly reduce in vivo hepatotoxicity and improve the therapeutic index. These results suggest that application of these novel nucleotide chemistries could lead to best-in-class anti-HBV ASOs. Currently, novel ASO therapeutics using this approach are being advanced into clinical trials for CHB.

Financial disclosure: All authors are current or former employees of Aligos Therapeutics, Inc.