S-antigen Transport-inhibiting Oligonucleotide Polymers, (STOPS™) Sequester Cellular Proteins to Reduce Hepatitis B Virus S-antigen Expression and Increase Its Proteasomal Degradation

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

Poster # 845

Abstract

Functional cure of chronic hepatitis B (CHB) requires eliminating the hepatitis B virus surface antigen (HBsAg) which can suppress the patient's antiviral immune response. Nucleic acid polymers can reduce serum HBsAg levels in CHB patients.[1-4] STOPS are phosphorothioated single-stranded oligonucleotides containing novel chemistries that can significantly reduce HBsAg produced by the hepatitis B virus (HBV)-infected hepatocytes *in vitro* and inhibit HBV infection. The STOPS molecule ALG-010133 is currently being studied in a Phase 1 clinical trial in CHB patients. However, the mechanism of action of how STOPS function has not been well described.

Objective

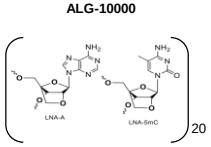
We seek to elucidate how STOPS molecules inhibit HBV replication and HBsAg levels.

Methods

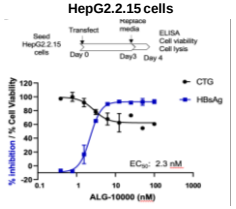
STOPS and siRNAs were transfected into HepG2.2.15 cells that contain integrated HBV genomes using Lipofectamine RNAiMax. Proteins from HepG2.2.15 cell lysates that bind STOPS were identified using affinity chromatography, tandem mass spectrometry, and searches of the human proteome database. HBsAg and other HBV proteins were quantified using ELISA and Western blotting. HBV nucleic acids were quantified using a Quantigene assay. Ubiquitination was detected by ELISA using a monoclonal antibody that was conjugated to horseradish peroxidase. Segments of the HBV genome were cloned into the vector pCDNA3.1. Recombinant HBsAg production was in transfected HEK 293 cells and detected by ELISA.

STOPS molecule, ALG-10000 can inhibit HBV infection and replication

ALG-10000

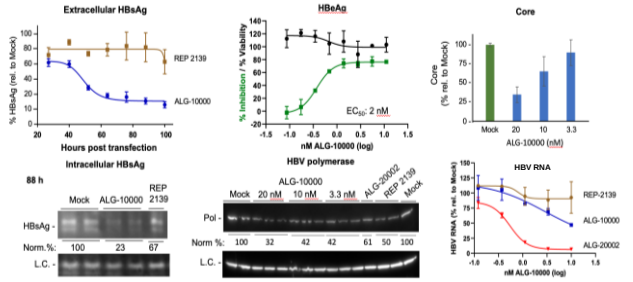


HepG2.2.15 cells



	EC_{50} (nM)	CC_{50} (nM)
HBV-infected HepG2-NTCP cells	4	> 1000
HBV-infected primary human hepatocytes	10	> 100

STOPS molecule ALG-10000 inhibits multiple HBV proteins and HBV RNA



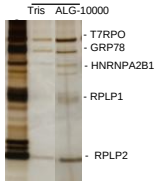
ALG-10000 binds host factors that function in gene expression and protein production

ALG-10000 does not bind HBV proteins or activate innate immune receptors RIG-I or TLR3. We hypothesize that it inhibits HBV infection through interaction with host factors

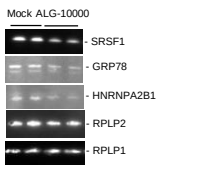
Top proteins that bind an ALG-10000 affinity resin

Protein	Gene Name	# peptides
Nucleophosmin	NPM1	7
Histone H4	HIST1H4A	6
Endoplasmic reticulum chaperone BiP	GRP78/BIP/HSPA5	5
Histone H2B	HIST1H2BK	5
Histone H2AX	H2AFX	4
Nuclease-sensitive element-binding protein	1YBX1	4
Nucleolin	NCL	4
Peroxiredoxin 1	PRDX1	4
Protein-lysine 6-oxidase	LOX	3
Dermicidin	DCD	3
Small nuclear ribonucleoprotein Sm D1	SNRPD1	2
A-kinase anchor protein 11	AKAP11	2
Histone H3	H3F3A	2
Heterogeneous nuclear riboprotein A2B1	HNRNPA2B1	1
Calmodulin 2	CALM2	1
Serine/arginine-rich splicing factor 1	SRSF1	1
Protein SETSIP	SETSIP	1
Actin, cytoplasmic 1	ACTB	1
Prelamin A/C	LMNA	1
Thioredoxin	TXN	1
60S acidic ribosomal protein P2	RPLP2	1
ATP synthase-coupling factor 6, mitochondrial	ATP5PF	1

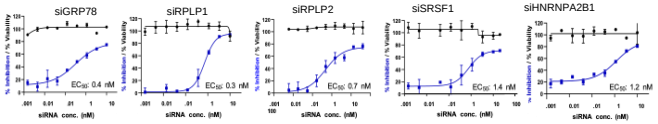
Recombinant HFs can bind ALG-10000 Resin



ALG-10000 reduces the levels of bound cellular proteins in cells

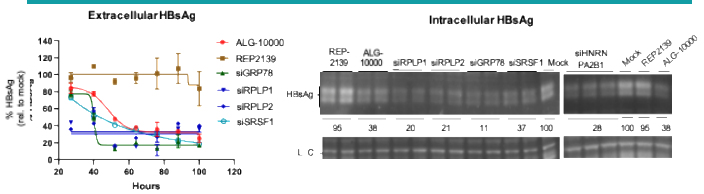


siRNAs knockdown of 5 HFs reduced HBsAg levels in HepG2.2.15 cells without cytotoxicity

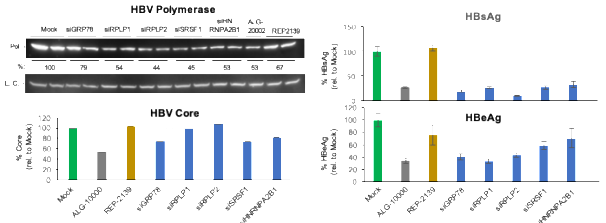


SRSF1: Serine-Arginine Splicing Factor.[4]
HNRNPA2B1: Heterogeneous ribonucleoprotein A2B1.[6]
RPLP1/2: Ribosomal protein lateral stalk proteins 1 and 2.[5]
GRP78: Membrane protein chaperon; Master regulator of the unfolded proteins response.[7]

Knockdowns of all 5 host factors inhibit both extracellular and intracellular HBsAg

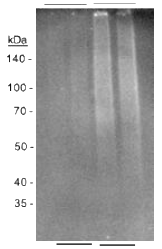


The 5 host factors function in the production of multiple HBV proteins and HBV RNA

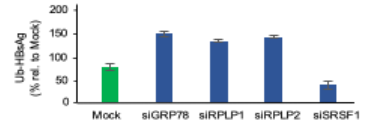


GRP78, RPLP1/2 are needed to stabilize HBsAg from proteosome-mediated degradation

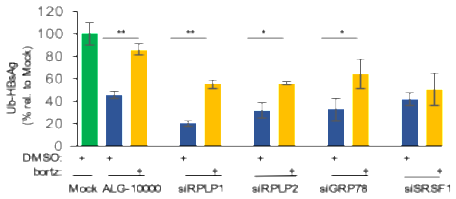
ALG-10000 increases the polyubiquitination of the HBsAg



Knockdowns of GRP78, RPLP1/2 increase ubiquitination of HBsAg

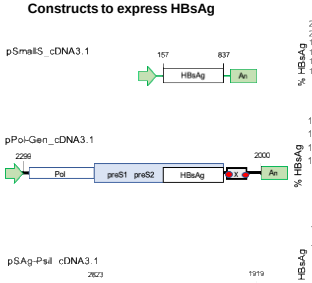


The proteosome inhibitor bortizimib can decrease HBsAg degradation

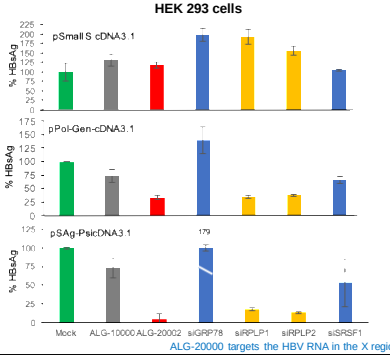


HBV RNA sequences are required for RPLP1 and RPLP2 to direct HBsAg production

Constructs to express HBsAg



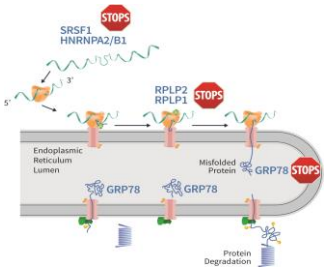
HEK 293 cells



ALG-20000 targets the HBV RNA in the X region

Conclusions

STOPS inhibit HBV replication and HBsAg production by sequestering cellular factors needed to express and properly fold HBsAg. Reduced levels of the cellular factors results in reduced HBsAg expression and increased ubiquitination and degradation of HBsAg. Several host factors require HBV RNA to act.



Acknowledgements

We thank R. Vaughan for mass spectrometric analyses. We thank M. Burke for generating a model for the mechanism of action of STOPS.

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Disclosures

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