

Supplementary Materials for

Preclinical Antiviral and Safety Profiling of the HBV RNA Destabilizer AB-161

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Table S1. HBV genotype coverage of AB-161.

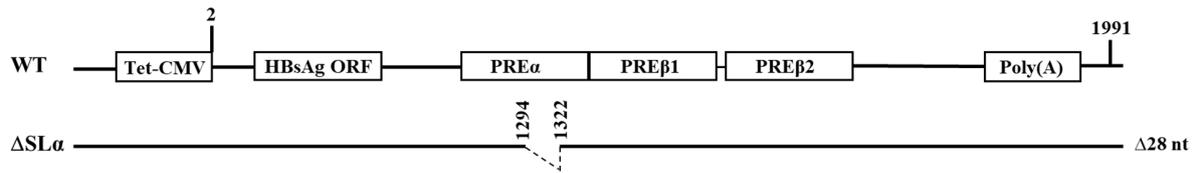
HBV genotype	HBsAg EC ₅₀ [nM]	CC ₅₀ [μM]
A	3.0 ± 0.8	>50
B	2.6 ± 1.4	>50
C	2.3 ± 0.6	>50
D	4.5 ± 2.8	>50
E	2.5 ± 0.9	>50
F	3.3 ± 2.6	>50
H-1	2.4 ± 0.5	>50
H-2	2.1 ± 0.9	>50

Data represent average value and standard deviations from at least 3 independent experiments.

Table S2. Antiviral selectivity and cytotoxicity of AB-161.

Virus ¹	Family (genome)	Cell line (tissue origin)	EC ₅₀ [μM]	CC ₅₀ [μM]
HBV	<i>Hepadnaviridae</i> (dsDNA)	HepG2.2.15 (human liver)	0.0023	>30
HCV	<i>Flaviviridae</i> (+ssRNA)	Huh-7 (human liver)	>30	>30
DENV2	<i>Flaviviridae</i> (+ssRNA)	BHK21 (hamster kidney)	>30	>30
WNV	<i>Flaviviridae</i> (+ssRNA)	Vero76 (monkey kidney)	>30	>30
HCMV	<i>Herpesviridae</i> (dsDNA)	MRC-5 (human lung)	>30	>30
HSV-1/HSV-2	<i>Herpesviridae</i> (dsDNA)	MRC-5 (human lung)	>30	>30
IAV	<i>Orthomyxoviridae</i> (-ssRNA)	MDBK (bovine kidney)	>30	>30
RSV	<i>Paramyxoviridae</i> (-ssRNA)	HEp2 (human epithelial)	>30	>30
HRV	<i>Picornaviridae</i> (+ssRNA)	HEp2 (human epithelial)	>30	>30
HIV	<i>Retroviridae</i> (+ssRNA)	CEM-22 (human T lymphoblast)	>30	>30

Abbreviations: HBV = hepatitis B virus; HCV= hepatitis C virus; DENV2 = dengue virus type 2; WNV = West Nile virus; HCMV = human cytomegalovirus; HSV-1/HSV-2 = herpes simplex virus-1/herpes simplex virus-2; IAV = influenza A virus; HRV = human rhinovirus; RSV = respiratory syncytial virus; HIV = human immunodeficiency virus.



Plasmid	AB-161 HBsAg EC ₅₀ [nM]
WT	2.5 ± 2.6
ΔSLα	>100

Figure S1. Stem-loop alpha sequence within the HBV PRE is critical for AB-161 antiviral activity. **(Top)** Schematic representations of plasmids encoding the open reading frame of HBsAg and either wild type (WT) or SLα deleted (ΔSLα) PRE sequence. **(Bottom)** Huh-7 cells transfected with WT or ΔSLα plasmids were treated with varying concentrations of AB-161. Levels of HBsAg were monitored to determine the effect of AB-161 against WT or the stem-loop alpha deletion mutant.

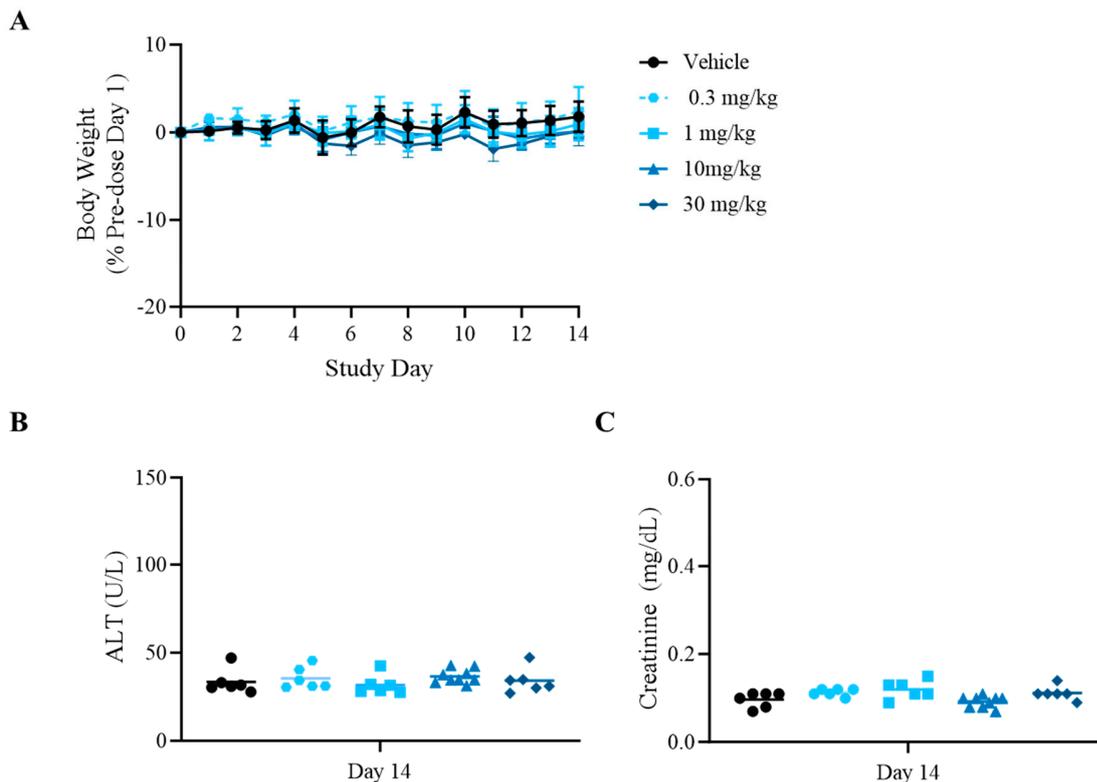


Figure S2. AB-161 once-daily dosing is well-tolerated in AAV-HBV mice. **(A)** AAV-HBV mice received once-daily oral doses of AB-161 or vehicle control from Days 0 to 13. Data represent body weights relative to pre-dose baseline levels ($n = 6-9$) of group mean \pm standard deviation. **(B)** Alanine aminotransferase (ALT) and **(C)** creatinine levels were measured in serum as markers of liver and kidney function, respectively. Data represent group mean ($n = 6-9$) \pm standard deviation.