Antiviral Characterization of a Next Generation Chemical Series of HBV Capsid Inhibitors *In Vitro* and *In Vivo*

Nagraj Mani[1], Andrew G. Cole[1], Janet R. Phelps[1], Cory Abbott[1], Andrzej Ardzinski[1], Jeff Bechardi[1], Robbin Burns[1], Tim Chiu[1], Andrea Cuconati[1], Bruce D. Dorsey[1], Ellen Evangelista[1], Kristi Fan[1], Laurel Fu[1], Fang Guo[1], Haitao Guo[2], Troy O. Harasym[1], Agnes Jarosz[1], Salam Kadhim[1], Steven G. Kultgen[1], Kaylyn Kwak[1], Amy C.H. Lee[1], Alice H. Li[1], Sara Majeski[1], Kevin McClintock[1], Angela Miller[1], Chris Pasetka[1], Stephen P. Reid[1], Rene Rijnbrand[1], Alexander Shapiro[1], Holly M. Steuer[1], Kim Stever[1], Sunny Tang[1], Xiaowei Teng[1], Xiaohe Wang[1], Hu Zhang[2], Michael J. Sofia[1]

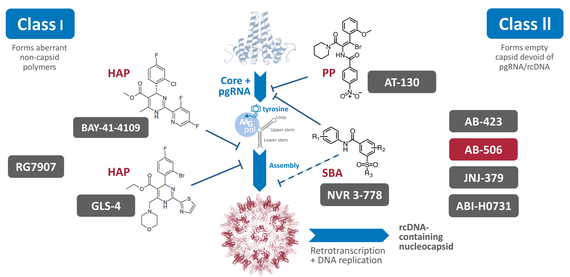
1. Arbutus Biopharma, Burnaby, BC, Canada and Warminster, PA, United States; 2. Indiana University, Indianapolis, IN, United States.

AASLD THE LIVER MEETING® 2017 WASHINGTON, DC OCTOBER 20-24 #953

Arbutus BIOPHARMA *Curing Chronic Hepatitis B*

BACKGROUND

- Hepatitis B virus (HBV) replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA)
- Proper assembly of HBV nucleocapsid is essential for viral genome relaxed circular DNA (rcDNA) synthesis, infectious virion production and maintenance of a nuclear covalently closed circular DNA (cccDNA) pool
- The capsid assembly process thus represents a *bona fide* antiviral target, and constitutes a novel mechanism that is distinct from the nucleos(t)ide analogues currently available for clinical use
- Interfering with HBV capsid assembly with small molecule inhibitors has been shown to translate into antiviral activity *in vitro* and *in vivo* (Cole, 2016)



HAP: heteroaryldihydropyrimidines; | SBA: sulfamoylbenzamides; | PP: = phenylpropenamides

Figure 1: HBV capsid assembly pathway and examples of capsid inhibitors.

OBJECTIVES

Characterize the *in vitro* and *in vivo* antiviral activities of a next generation chemical series of potent small-molecule inhibitors of HBV capsid assembly.

MATERIALS AND METHODS

- Compounds were tested in a biochemical assay of capsid assembly as described previously (Zlotnick, 2007)
- The ability of compounds to bind and thermally stabilize core protein was determined in thermal shift assays using differential scanning fluorimetry
- X-ray crystallography studies were conducted to determine the binding mode of compounds to core protein Cp-Y132A mutant
- Antiviral activity was determined in different cell culture models of HBV using branched DNA, quantitative PCR, and AlphaLISA®assays to measure effects on rcDNA or secreted e-antigen
- Activity against HBV genotypes and nucleoside analog inhibitor-resistant (Nuc^R) variants was determined using a transient transfection assay system
- Cytotoxicity of compounds was evaluated in various cell lines using CellTiter-Glo® or MTT assay
- Antiviral activity against viruses of various families was determined using cell culture assays
- Pharmacokinetic profiles of the compound were determined in CD-1 mice, SD rats, and Beagle dogs
- The *in vivo* antiviral activity was assessed in a hydrodynamic injection (HDI) HBV mouse model utilizing pHBV1.3 (Guidotti 1995). Test articles were administered orally for 7 days starting on Day 0, AB-506 and vehicle twice daily and ETV once daily. HBV DNA was measured using qPCR. Reported liver HBV DNA values are vector-subtracted

RESULTS

Table 1: *In vitro* antiviral activities of next generation capsid inhibitors:

Compound	HepDE19 (rcDNA_bDNA) (μM)			HepBHAe82 (HBeAg AlphaLISA) (μM)			HepG 2.2.15 (HBV DNA qPCR) (μM)	
	EC_{50}	EC_{90}	CC_{50}	EC_{50}	EC_{90}	CC_{50}	EC_{50}	CC_{50}
Compound A	0.11	0.45	>25	0.07	0.28	>25	0.08	>10
Compound B	0.06	0.27	>25	0.03	0.14	>25	0.04	>10
Compound C	0.04	0.14	15	0.01	0.07	22	0.04	>10
AB-506	0.07	0.27	>25	0.04	0.20	>25	0.04	>10

Table 2: Antiviral activity against HBV genotypes A through D and potency of AB-506 against Nuc^R variants:

HBV Nuc^R Variant	HBV DNA qPCR AB-506 (EC_{50} μM)
rtM204I	0.059
rtM204I + V173L	0.038
rtM204I + S202G	0.052
rtM204V + L180M	0.055
rtM204I + S202G + M250V	0.061
WT, GtD	0.040

HBV Genotype	HBV DNA qPCR AB-506(EC_{50} μM)
A1	0.008
A2	0.023
B1	0.017
B2	0.020
C1	0.015
C2	0.009
D	0.040

- No cross-resistance with Nuc^R variants. Consistent with their distinct mechanism of action.
- AB-506 shows activity against the most prevalent HBV genotypes globally.

Table 3: Antiviral selectivity of AB-506:

Virus	Family	Genome	AB-506 EC_{50} (μM)	AB-506 CC_{50} (μM)	Host Cell Line
HCV	*Flaviviridae*	(+) ssRNA	>30	>30	Huh7
WNV	*Flaviviridae*	(+) ssRNA	>30	>30	VERO
RSV	*Paramyxoviridae*	non-segmented (-) ssRNA	>30	>30	HEp2
IFA	*Orthomyxoviridae*	segmented (-) ssRNA	>30	>30	MDCK
HIV	*Retroviridae*	ssRNA to DNA	>30	>30	CEMSS
HSV1&2	*Herpesviridae*	dsDNA	>30	>30	VERO
HCMV	*Herpesviridae*	dsDNA	>30	>30	MRC5
DENV	*Flaviviridae*	(+) ssRNA	>22	22	BHK21
HRV	*Picornaviridae*	(+) ssRNA	>30	>30	H1/HeLa

HCV = Hepatitis C Virus; WNV = West Nile Virus; RSV = Respiratory Syncytial Virus; IFA = Influenza A Virus; HIV = Human Immunodeficiency Virus; HSV = Herpes Simplex Virus; hCMV =Human Cytomegalovirus; DENV = Dengue Virus; HRV = Human Rhinovirus

- No significant inhibition of a panel of RNA & DNA viruses demonstrating high selectivity for HBV.

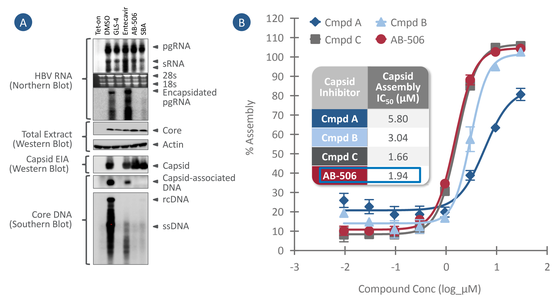


Figure 2: A) AB-506 forms empty capsids devoid of pgRNA or rcDNA in HepAD38 cells; B) Next generation capsid inhibitors accelerate capsid assembly reaction *in vitro*.

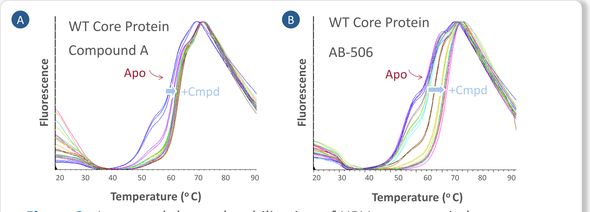


Figure 3: Increased thermal stabilization of HBV core protein by next generation capsid inhibitors. A) Compound A and B) AB-506 binding increases thermal stability of WT core protein by up to 2°C and 6°C, respectively.

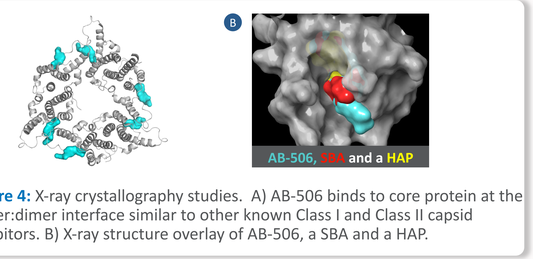
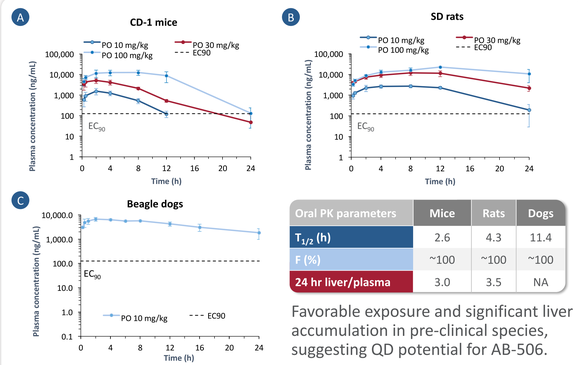


Figure 4: X-ray crystallography studies. A) AB-506 binds to core protein at the dimer:dimer interface similar to other known Class I and Class II capsid inhibitors. B) X-ray structure overlay of AB-506, a SBA and a HAP.



Oral PK parameters	Mice	Rats	Dogs
$T_{1/2}$ (h)	2.6	4.3	11.4
F (%)	~100	~100	~100
24 hr liver/plasma	3.0	3.5	NA

Favorable exposure and significant liver accumulation in pre-clinical species, suggesting QD potential for AB-506.

Figure 5: Pharmacokinetics of AB-506 in A) mice, B) rat, and C) dog.

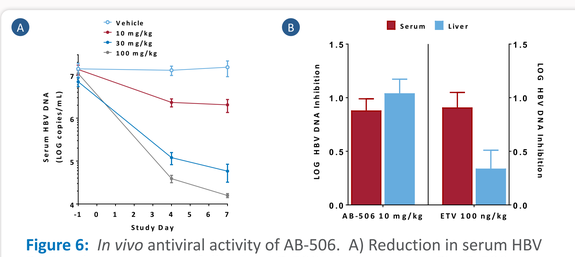


Figure 6: *In vivo* antiviral activity of AB-506. A) Reduction in serum HBV DNA is dose responsive following AB-506 administration. B) AB-506 surpassed ETV at inhibiting liver HBV DNA, at dosages where the serum HBV DNA inhibition was equivalent (data relative to vehicle at Day 7)

CONCLUSIONS

- AB-506 is a next generation highly selective HBV capsid inhibitor
- *In vitro* AB-506:
  - showed potent inhibition of HBV replication in cell culture models
  - bound at the dimer:dimer interface of core protein in X-ray crystallography studies
  - inhibited pgRNA encapsidation in HepAD38 cells
  - accelerated rate of capsid assembly in a biochemical assay
  - conferred increased thermal stability to core protein indicating improved target engagement compared to first generation capsid inhibitors
- Dosing performed in multiple species suggest QD potential and significant liver concentrations achieved
- AB-506 showed potent *in vivo* activity in a HDI mouse model of HBV
- AB-506 is being evaluated for advancement into clinical development

REFERENCES

- Cole AG. 2016. Modulators of HBV capsid assembly as an approach to treating hepatitis B virus infection. ***Curr. Opin. Pharmacol.*** **30:**131-137.
- Campagna MR, Liu F, Mao R, Mills C, Cai D, Guo F, Zhao X, Ye H, Cuconati A, Guo H, Chang J, Xu X, Block TM, Guo JT. 2013. Sulfamoylbenzamide derivatives inhibit the assembly of hepatitis B virus nucleocapsids. ***J. Virol.*** **87(12):**6931-6942.
- Guidotti LG, Matzke B, Schaller H, Chisari FV. 1995. High-level hepatitis B virus replication in transgenic mice. ***J. Virol.*** **69(10):**6158-6169.
- Hu Y, Zhu W, Tang G, Mayweg AV, Yang G, Wu JZ, Shen HC. 2013. Novel therapeutics in discovery and development for treatment of chronic HBV infection. ***Ann. Med. Chem. Rep.*** **48:**265-281
- Zlotnick A, Lee A, Bourne CR, Johnson JM, Domanico PL, Stray SJ. 2007. *In vitro* screening for molecules that affect virus capsid assembly (and other protein association reactions). ***Nat. Protoc.*** **2(3):**490-498.

CONTACT INFORMATION AND DISCLOSURES

NAGRAJ MANI, Ph.D., Sr. Principal Scientist,

- Arbutus Biopharma Inc., 701 Veterans Circle, Warminster, PA 18974.
- Email: nmani@arbutusbio.com
- Tel: 1-267-420-2712;
- Authors affiliated with Arbutus Biopharma are employees and may own company stock

WEBSITE:

www.arbutusbio.com