

In Vitro and In Vivo Antiviral Activities of AB-423 a Potent Small Molecule Inhibitor of Hepatitis B Virus Capsid Assembly

Nagraj Mani^{1*}, Andrew G. Cole¹, Janet R. Phelps², Andrzej Ardzinski¹, Kyle D. Cobarrubias², Andrea Cuconati¹, Bruce D. Dorsey¹, Ellen Evangelista², Troy O. Harasym², Salam Kadhim², Amy C.H. Lee³, Hongyan Liang³, Alice H.L. Li², Kevin D. McClintock², Sara A. Majeski², Stephen P. Reid², Rene Rijnbrand¹, Sunny Tang², Xiaohu Wang¹, and Michael J. Sofia¹

Arbutus Biopharma Inc., ¹Doylestown, PA, USA and ²Burnaby, BC, Canada; ³Baruch S. Blumberg Institute, Doylestown, PA, USA



BACKGROUND

- Hepatitis B virus replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA).
- Proper assembly of HBV nucleocapsid is essential for viral genome (rcDNA) synthesis, infectious virion production and maintenance of a nuclear cccDNA pool.
- The capsid assembly process thus represents a *bonafide* 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*.

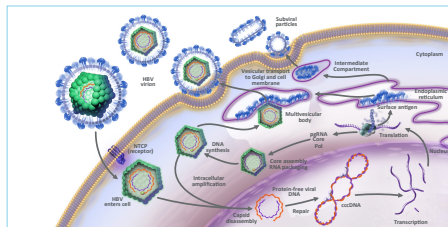


Fig 1. HBV life cycle

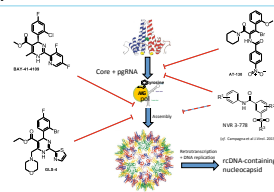


Fig 2. Inhibition of HBV capsid assembly and pgRNA encapsidation are well validated targets

OBJECTIVES

- Characterize the *in vitro* and *in vivo* antiviral activities of AB-423, a potent small-molecule inhibitor of HBV capsid assembly.

MATERIALS & METHODS

- AB-423 was tested in a biochemical assay of capsid assembly as described previously (Zlotnick 2007).
- Antiviral activity of AB-423 was determined in different cell culture models of HBV using dot blot, branched DNA or quantitative PCR assays to measure effects on HBV precore RNA secreted e-antigen or rcDNA synthesis.
- Pan-genotypic activity and activity against Nuc^R variants was determined using a transient transfection assay system.
- Cytotoxicity of AB-423 was evaluated in various cell lines using CellTiter-Glo or MTT assay.
- Antiviral activity of AB-423 against viruses of various families was determined using different cell culture assay systems.
- In vitro* combination studies were conducted in HBV cell culture models at different compound concentrations in a checkerboard format and analyzed using the MacSynergy II program (Prichard and Shipman 1990).
- The *in vivo* antiviral activity of AB-423 as monotherapy and in combination with Entecavir was assessed in a hydrodynamic injection HBV mouse model.

RESULTS

Table 1. AB-423 is a potent inhibitor of HBV replication *in vitro*

Potency	EC ₅₀	EC ₅₀	CC ₅₀	Endpoint
DESHAe82 (μM)	0.25	1.17	>10	eAg/EUSA
AML12-HBV10 (μM)	0.15	ND	>10	rcDNA/Dot Blot
AML12-HBV10 (μM)	0.28	1.96	>10	rcDNA/bDNA
HepDE19 (μM)	0.34	0.63	>10	rcDNA/bDNA

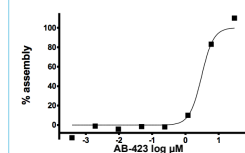


Fig 3. AB-423 misdirects capsid assembly in a biochemical assay. In a biochemical capsid assembly assay, AB-423 misdirects capsid assembly with an IC₅₀ value of 3 μM.

Table 2. AB-423 has pan-genotype activity

Genotype	AB-423 EC ₅₀ (μM)
A-1	0.057
A-2	0.089
B-1	0.039
B-2	0.091
C-1	0.052
C-2	0.055
D	0.195

RESULTS

Table 3. AB-423 shows potent activity against Nuc^R variants

HBV Variant	AB-423 EC ₅₀ (μM)	ETV EC ₅₀ (μM)	LAM EC ₅₀ (μM)
rtM204I	0.192	ND	>100
rtM204I+V173L	0.151	ND	>100
rtM204I+S202G	0.190	10.7	ND
rtM204V+L180M	0.175	ND	>100
rtM204I+S202G+M250V	0.235	9.0	ND
U95551 (WT, GtD)	0.105	0.002	0.03

ETV = Entecavir; LAM = Lamivudine

Table 4. AB-423 is a selective inhibitor of HBV

Virus	Family	Genome	EC ₅₀ (μM)	CC ₅₀ (μM)	Host Cell Line
HCV	Flaviviridae	(+) ssRNA	11.2	>30	Huh7
WNV	Flaviviridae	(+) ssRNA	>30	>30	VERO
RSV	Paramyxoviridae	non-segmented (-) ssRNA	19.2	>30	Hep2
IFA	Orthomyxoviridae	segmented (-) ssRNA	>30	>30	MDCK
HIV	Retroviridae	ssRNA to DNA	>30	>30	CEMSS
HSV	Herpesviridae	dsDNA	>30	>30	VERO
hCMV	Herpesviridae	dsDNA	>30	>30	MRC5
DENV	Flaviviridae	(+) ssRNA	>30	>30	Huh7
HRV 1A	Picornaviridae	(+) ssRNA	7.18	>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

Table 5. No antagonism when AB-423 was combined with Entecavir *in vitro*

Inhibitor A	Inhibitor B	Cell Culture Model	Conclusion*
AB-423	ETV	AML12-HBV10 (rcDNA)	Additive
AB-423	ETV	DESHAe82 (precore RNA)	Synergy

*MacSynergy II Analysis; Bliss Independence Model; Prichard and Shipman 1990.

CONCLUSIONS

- AB-423 is a potent, highly selective inhibitor of HBV replication through a block of encapsidation.
- In vitro* AB-423 showed:
 - additive to synergistic antiviral activity in combination with ETV.
 - potent activity against HBV Nuc^R variants.
 - no significant activity against unrelated viruses.
- Dosing performed in multiple species, mouse data indicates significant liver concentrations achieved.
- AB-423 showed potent *in vivo* activity in a HDI mouse model of HBV (alone or in combination with ETV).
- AB-423 is being evaluated for advancement into clinical development.

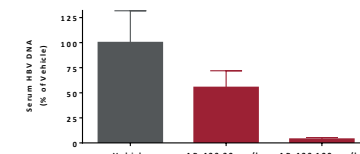


Fig 4. AB-423 Shows Potent *In Vivo* Antiviral Activity in a Mouse Model of HBV Infection. NOD.CB17-Prkdc^{scid} mice express HBV from a 1.3-fold overlength copy of a genotype D genome that had been administered to the liver via hydrodynamic injection (HDI) of plasmid pHBV1.3 (Guidotti 1995). Subsequently, the animals were given oral doses of vehicle or AB-423 twice a day for 7 days. On Day 7, serum HBV DNA was measured by QPCR and individual animal changes calculated against pre-dose values on Day 0. Data shown as mean ± SEM (n=5-6).

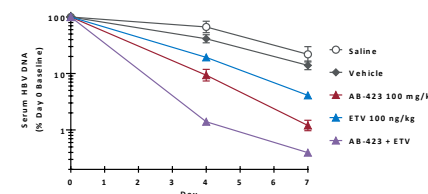


Fig 5. *In Vivo* Demonstration of Combination Treatment of AB-423 with a Nucleoside Analog. The HDI mouse model of HBV is described in Figure 4. Saline, vehicle and AB-423 were orally administered twice a day for 7 days. The nucleoside analog Entecavir (ETV) was orally administered once a day for 7 days. Serum HBV DNA was measured by QPCR and results were expressed as a percentage of individual animals' Day 0 pre-dose values. Data shown as mean ± SEM (n=7-8).

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Contact Information:

Nagraj Mani, Ph.D., Principal Scientist, Arbutus Biopharma Inc., 3805 Old Easton Rd., Doylestown, PA 18902.
Email: nmmani@arbutusbio.com; Tel: 416-913-1127