Identification and Characterization of AB-452, a Potent Small Molecule HBV RNA Destabilizer *In Vitro* and *In Vivo*

Dimitar Gotchev[1], Min Gao[1], Chris Moore[1], Andrew Kondratowicz[2], Cory Abbott[2], Lauren Bailey[1], Yingzhi Bi[1], Robbin Burns[2], Shuai Chen[1], Tim Chiu[2], Bruce D. Dorsey[1], Ellen Evangelista[2], Laurel Fu[2], David Fraser[2], Fang Guo[1], Troy O. Harasym[2], Richard Holland[2], Agnes Jarosz[2], Salam Kadhim[2], Ramesh Kakarla[1], Kaylyn Kwak[2], Amy C. H. Lee[2], Alice H. L. Li[2], Fei Liu[1], Sara Majeski[2], Kevin McClintock[2], Angela Miller[2], Dan Nguyen[1], Chris Pasetka[2], Luying Pei[2], Stephen P. Reid[2], Rene Rijnbrand[1], Alexander Shapiro[2], Sunny Tang[2], Xiaowei Teng[2], Lucy Wang[1], Mark Wood[2], Xin Ye[2], and Michael J. Sofia[1]

Arbutus Biopharma Inc., [1]Warminster, PA, USA and [2]Burnaby, BC, Canada

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BACKGROUND

- Expression of viral proteins, especially HBsAg, has been strongly associated with HBV persistence. High levels of serum HBsAg may impair B/T cell function *in vivo* by masking neutralizing antibodies and contributing to T cell exhaustion
- HBsAg seroconversion, an indicator of HBV cure, is always accompanied by HBsAg seroclearance
- Current approved treatment for HBV using nucleos(t)ides or interferon, can effectively suppress viral replication, but cures are rare
- Development of therapeutic agents targeting the expression of the HBV proteins, in particular HBsAg, can potentially provide much needed addition to treatment options for HBV cure

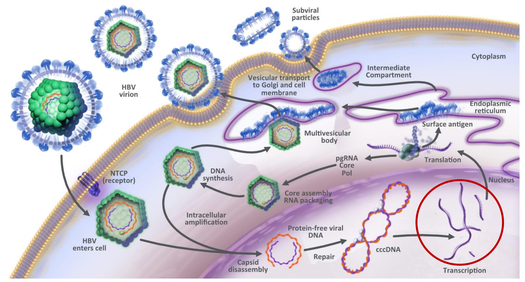

Figure 1: HBV life cycle and role of RNA destabilizers

OBJECTIVES

Characterize the *in vitro* and *in vivo* antiviral activities of AB-452, a potent small molecule that destabilizes HBV RNA.

MATERIALS AND METHODS

- Inhibition of HBsAg was determined in HepG2.2.15, Huh-7, primary human hepatocytes and HepG2/NTCP cells using a HBsAg immunoassay (microplate-based CLIA kits, Autobio Diagnosics Co., Zhengzhou, China)
- Antiviral activity was determined in different cell culture models of HBV using ELISA, quantitative PCR and Northern/Sothern analysis to measure effects on HBsAg, HBeAg, RNA or DNA
- Genotype activity was determined using a transient transfection assay system
- Cytotoxicity was evaluated in various cell lines using CellTiter-Glo, HCV replicon and cell protection assays
- Antiviral activity 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 combinations in a checkerboard format and analyzed using the MacSynergy II program[1]
- The *in vivo* antiviral activity as monotherapy was assessed in an AAV HBV mouse model[2]

RESULTS

Table 1: AB-452 is a potent inhibitor of HBV replication *in vitro*

Potency Model	EC_{50} (μM)	CC_{50} (μM)	Endpoint
HepG2.2.15*	0.0015	>50	HBsAg/ELISA
HepG2.2.15	0.0028	>50	HBeAg/ELISA
HepG2.2.15	0.0002	>50	HBV DNA/qPCR
PHH	0.0087	>1	HBsAg/ELISA
PHH	0.0088	>1	HBeAg/ELISA
HepG2/NTCP	0.0097	ND	HBsAg/ELISA
HepG2/NTCP	0.0036	ND	HBeAg/ELISA

* Human serum shift was 2x

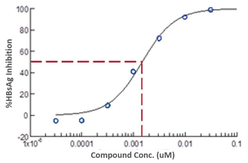

Figure 2: AB-452 is a potent HBsAg inhibitor in HepG2.2.15 cells with an EC_{50} value of 1.5 nM

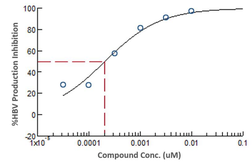

Figure 3: AB-452 is a potent inhibitor of HBV DNA in HepG2.2.15 cells with an EC_{50} value of 0.2 nM

Table 2: AB-452 has broad genotype activity

Genotype*	EC_{50} (μM)**
A	0.0013
B	0.0018
C	0.0020
D	0.0008

* Plasmids were verified by DNA sequencing (Huh-7 cells used in transfection)

** Readout HBsAg

Table 3: AB-452 is a selective inhibitor of HBV

Virus	Family	Genome	AB-452		Host Cell Line
			EC_{50} (μM)	CC_{50} (μM)	
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
HSV	*Herpesviridae*	dsDNA	>30	>30	VERO
hCMV	*Herpesviridae*	dsDNA	>30	>30	MRC5
DENV	*Flaviviridae*	(+) ssRNA	>30	>30	Huh7
HRV 1A	*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

Table 4: AB-452 shows additive to synergistic effects when combined with HBV LNP siRNA agents *in vitro*

Inhibitor A	Inhibitor B	Cell Culture Model	Conclusion*
AB-452	ARB-1467**	HEPG2.2.15 (HBsAg)	Additive
AB-452	ARB-1740**	HEPG2.2.15 (HBsAg)	Additive

*MacSynergy II Analysis; Bliss Independence Model[1]

**ARB-1467 and ARB-1740 are HBV LNP siRNA agents

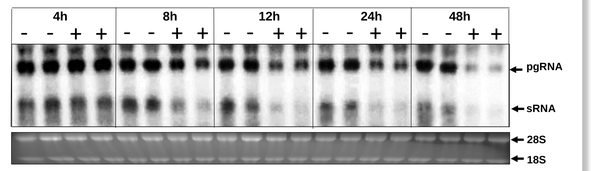

Figure 4: Time course of HBV RNA reduction by AB-452. HBV RNA reduction occurs at 4 to 8 hours after addition of 70 nM inhibitor in HepG2.2.15 cells.

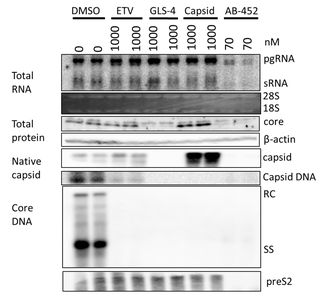

Figure 5: Multiple aspects of the HBV lifecycle affected by AB-452. HBV RNA reduction leads to interference in viral gene expression, DNA replication, and virion assembly in HepG2.2.15 cells.

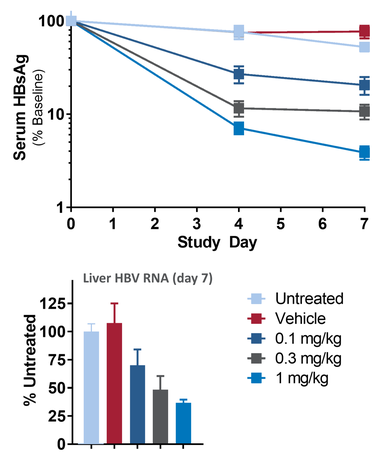

Figure 6: Administration of AB-452 for 1 week, PO, BID at 0.1, 0.3 and 1 mg/kg resulted in up to 1.4 log10 serum HBsAg reduction in a dose-dependent manner. This correlated well with liver HBV RNA levels. An immunocompetent mouse model of chronic HBV, infected with an AAV carrying a 1.2-fold overlength genome of genotype D was utilized and results are expressed as a percentage of individual animals' Day 0 pre-dose values. Data shown as mean ± SEM (n=5).

CONCLUSIONS

- AB-452 is a potent, highly selective inhibitor of HBV replication through destabilization of HBV RNA
- *In vitro* AB-452 showed:
  - additive to synergistic antiviral activity in combination with LNP siRNA agents
  - no significant activity against unrelated viruses
  - no apparent *in vitro* cytotoxicity
- AB-452 significantly inhibited both HBV replication and antigenemia in an immunocompetent AAV mouse model
- AB-452 is being evaluated for advancement into clinical development

REFERENCES

1. Prichard, M. N. and Shipman, C., Jr. A three-dimensional model to analyze drug-drug interactions. *Antiviral Res.* **1990**, *14*, 181.
2. Dion, S.; Bourgine, M.; Godon, O.; Levillayer, F.; Michel, M.-L. Adeno associated virus-mediated gene transfer leads to persistent hepatitis B virus replication in mice expressing HLA-A2 and HLA-DR1 molecules. *J. Virol.* **2013**, *87*, 5554.

CONTACT INFORMATION AND DISCLOSURES

DIMITAR GOTCHEV, Ph.D., Sr. Principal Scientist

- Arbutus Biopharma Inc., 701 Veterans Circle, Warminster, PA 18974
- Email: dgotchev@arbutusbio.com
- Tel: 1-267-332-6816
- Authors affiliated with Arbutus Biopharma are employees and may own company stock

WEBSITE:

www.arbutusbio.com