The HBV capsid inhibitor AB-423 exhibits a dual mode of action and displays additive/synergistic effects in *in vitro* combination studies

Rene Rijnbrand, PhD
VP Biology

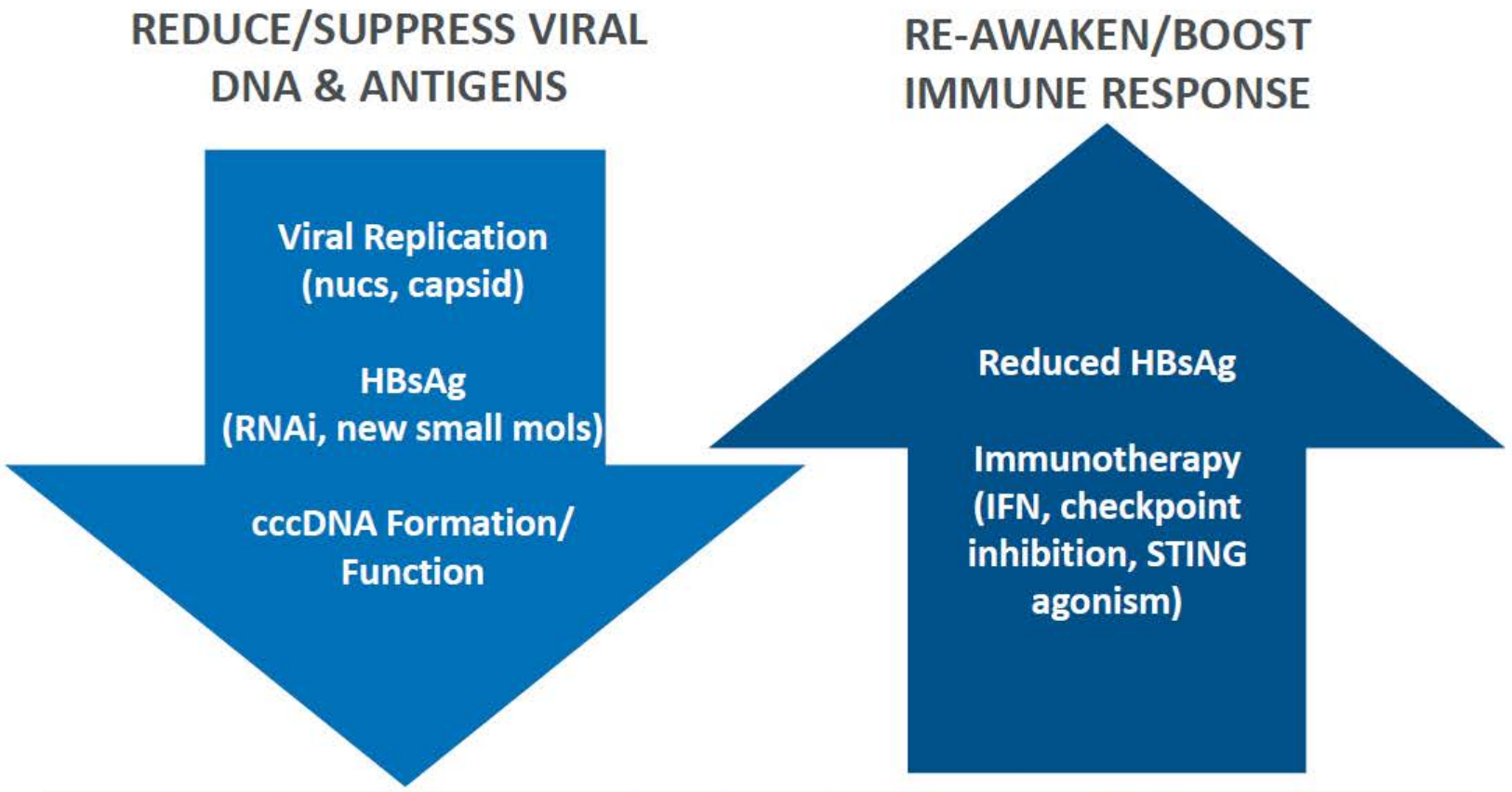
Nagraj Mani, Andrew G. Cole, Andrzej Ardzinski, Dawei Cai, Andrea Cuconati, Bruce D. Dorsey, Fang Guo, Haitao Guo, Ju-Tao Guo, Steven Kultgen, Amy C. Lee, Rene Rijnbrand, Nicholas M. Snead, Holly Steuer, Xiaohe Wang, Qiong Zhao, Michael J. Sofia

Arbutus Biopharma, Warminster, PA, USA and Burnaby, BC, Canada
Baruch S. Blumberg Institute, Doylestown, PA, USA
Indiana University, Indianapolis, IN, USA.

Disclosure Statement

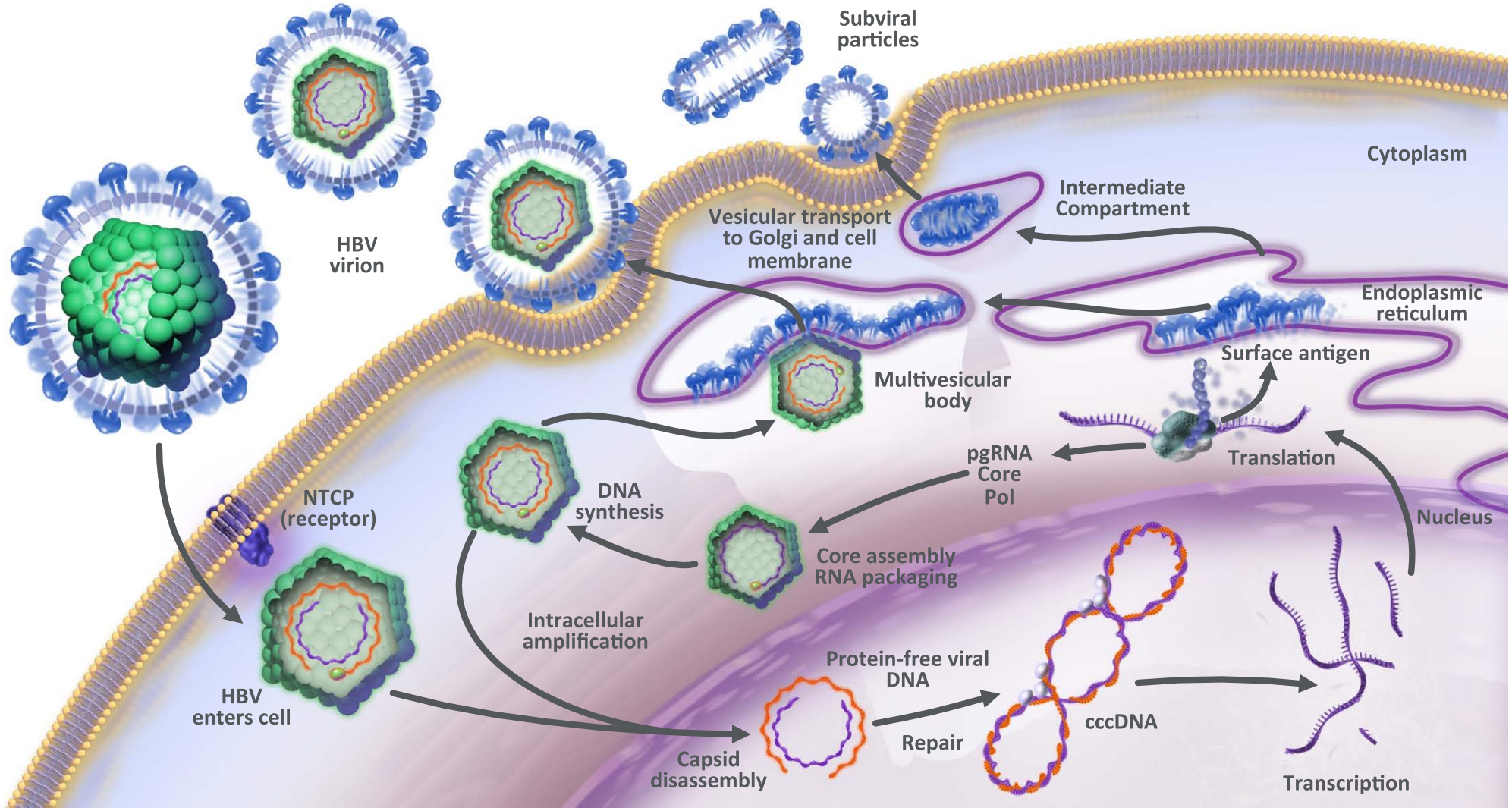
Employees of Arbutus Biopharma:
Nagraj Mani, Andrew G. Cole, Andrzej Ardzinski, Andrea Cuconati, Bruce D. Dorsey, Steven Kultgen, Amy C. Lee, Rene Rijnbrand, Nicholas M. Snead, Holly Steuer, Xiaohe Wang, and Michael J. Sofia

Key to Therapeutic Success



A combination approach to these key factors will drive cures

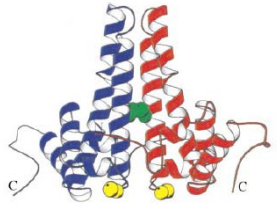
HBV Life Cycle



Capsid Assembly is a Validated Target

- Hepatitis B virus replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA) prior to rcDNA synthesis, virion production, and subsequent cccDNA synthesis.
- Assembly of HBV nucleocapsid is dependent on ordered folding of the viral capsid protein.
- Interfering with HBV capsid assembly with small molecule inhibitors has been shown to translate into antiviral activity *in vitro* and *in vivo* and constitutes a mechanism that is distinct from the nucleos(t)ide analogues currently available for clinical use.

Inhibition of HBV Capsid Assembly and pgRNA Encapsidation are Validated Targets

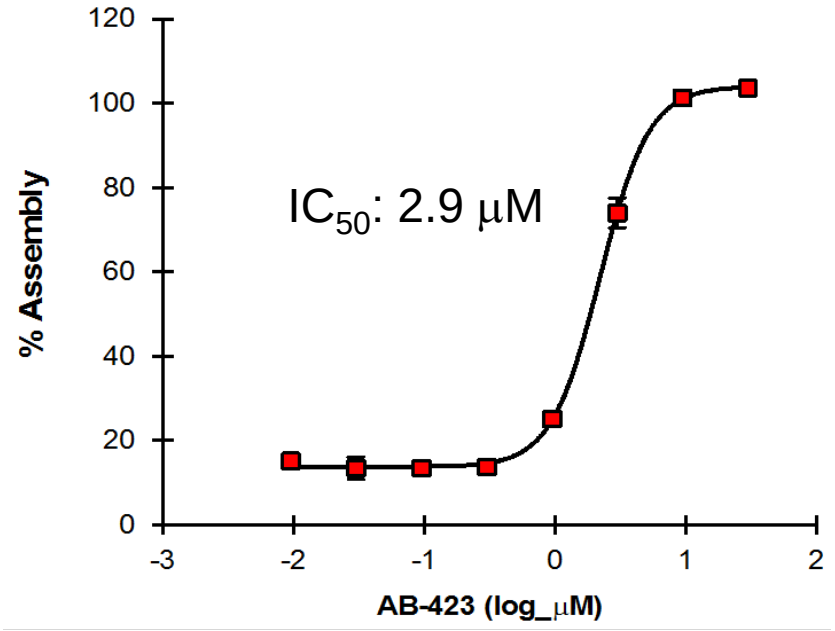


NVR 3-778

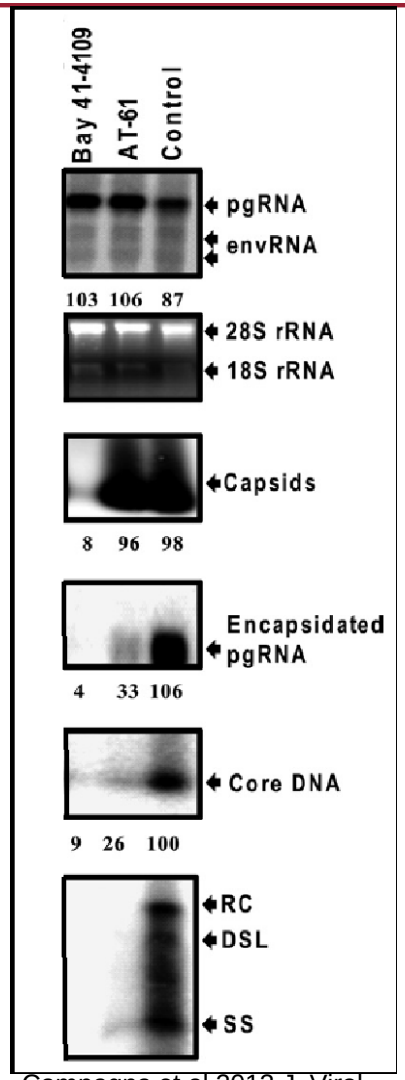
- A number of small molecules belonging to these two classes of core protein modulators have been described

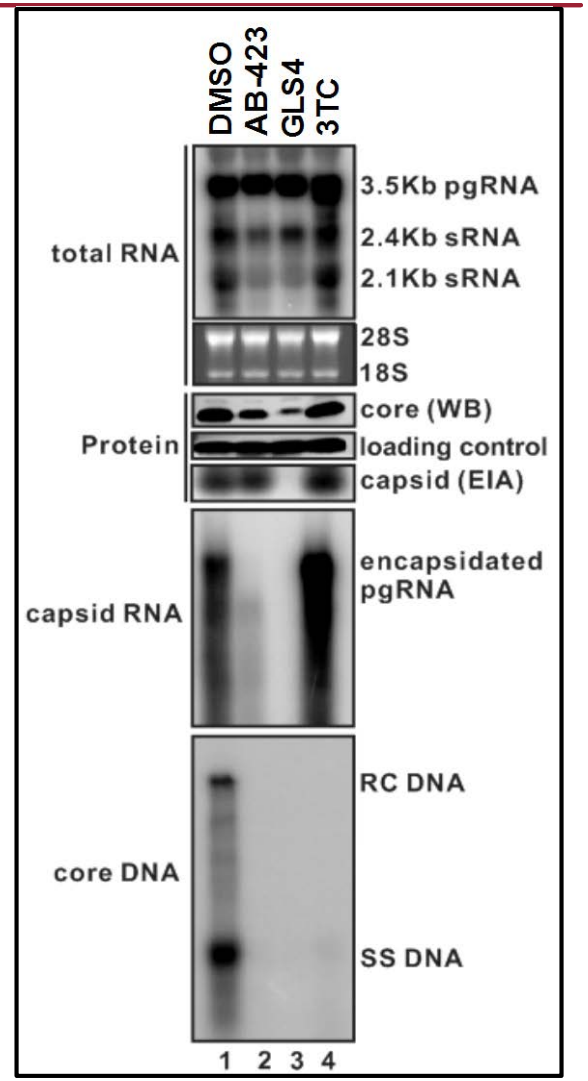
AB-423 Inhibits HBV pgRNA Encapsidation and Misdirects Capsid Assembly *In Vitro* and in Tissue Culture

- In a biochemical capsid assembly assay, AB-423 misdirects capsid assembly



- AB-423 inhibits pgRNA encapsidation in an HBV cell culture model system



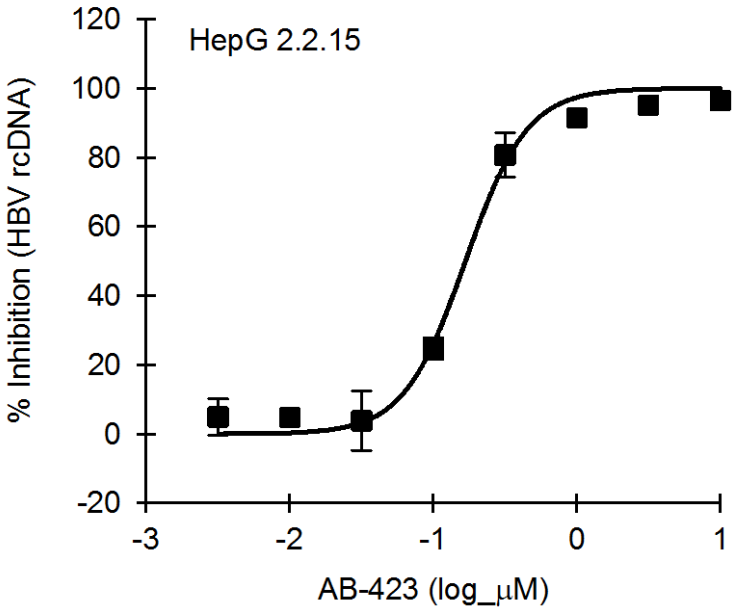


Campagna et al 2013 J. Virol

AB-423 is an Inhibitor of HBV Replication

	EC_{50} (µM)*	EC_{90} (µM)*	CC_{50} (µM)#	Assay
HepG 2.2.15	0.146± 0.024	0.993 ± 0.855	>10	(rcDNA/qPCR) human hepatoma cell line
HepDE19	0.262 ± 0.127	0.905 ± 0.332	>10	(rcDNA/bDNA) human hepatoma cell line
AML12-HBV10	0.263 ± 0.177	1.319 ± 1.076	>10	(rcDNA/bDNA) mouse hepatoma cell line
HepBHAe82	0.267 ± 0.135	1.246 ± 0.466	>10	(eAg/ELISA) human hepatoma cell line
PHH	0.078±0.031	0.333±0235	>10	(virion DNA/qPCR) Primary human hepatocytes

* EC_{50}/EC_{90} ± SD
# Highest concentration tested



AB-423 has Pan Genotypic Activity

- Most tissue culture systems represent gt D

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

- Activity maintained across gt A-D maintained within a 4-fold range, with gt A-C being more sensitive than gt D

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	9.042	ND
U95551 (WT, GtD)	0.105	0.002	0.03

- No cross-resistance with Nuc^{R} variants. Consistent with their distinct mechanisms of action.

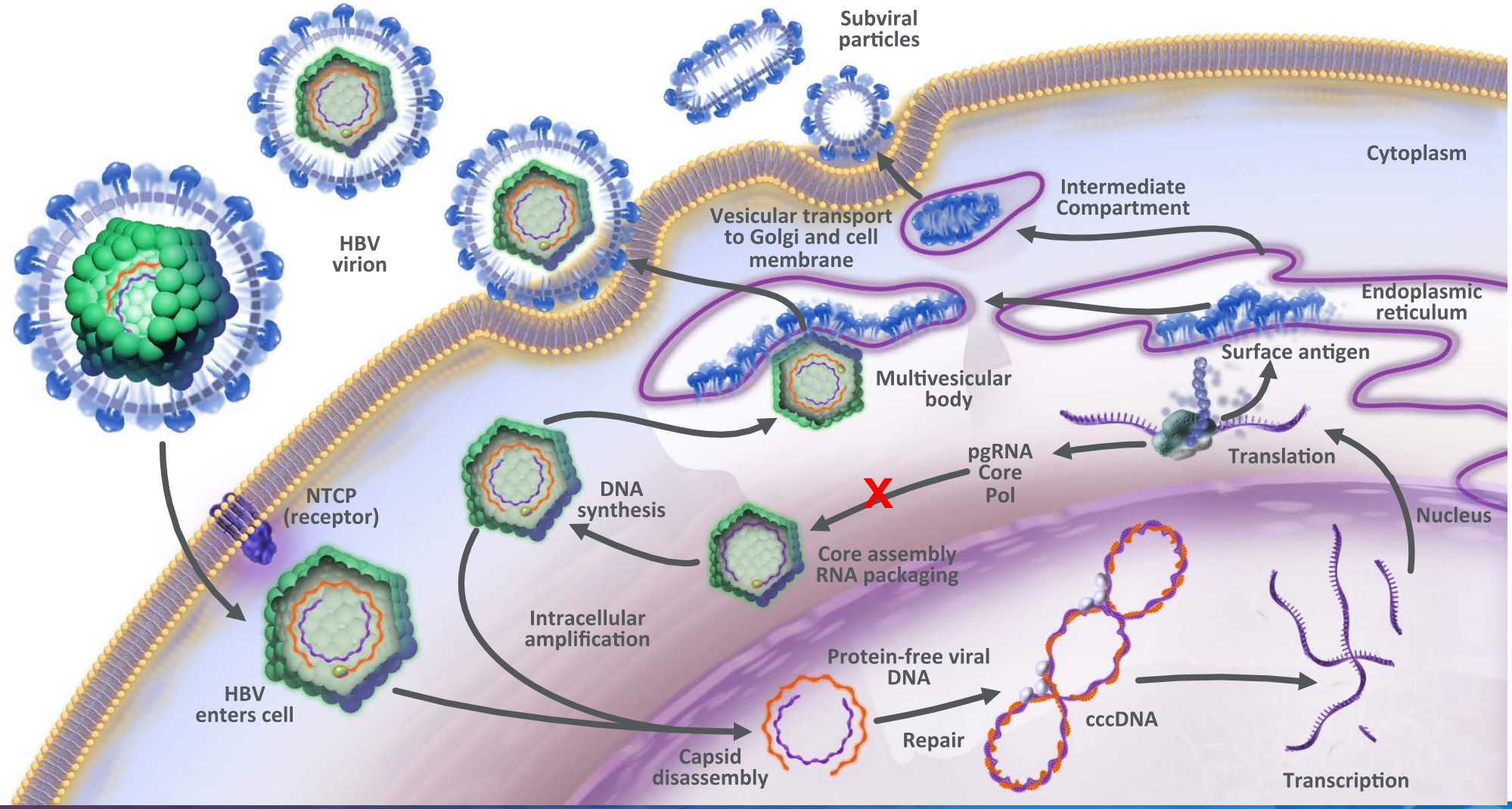
AB-423 is a Selective Inhibitor of HBV

(+)-RNA	(-)-RNA)	dsDNA	ssRNA to DNA
Flaviviridae	Orthomyxoviridae	Herpesviridae	Retroviridae
HCV	Influenza A	HSV	HIV
WNV		CMV	
DenV	Paramyxoviridae		
	RSV		
Picornaviridae			
HRV 1A			

AB-423 is a Selective Inhibitor of HBV

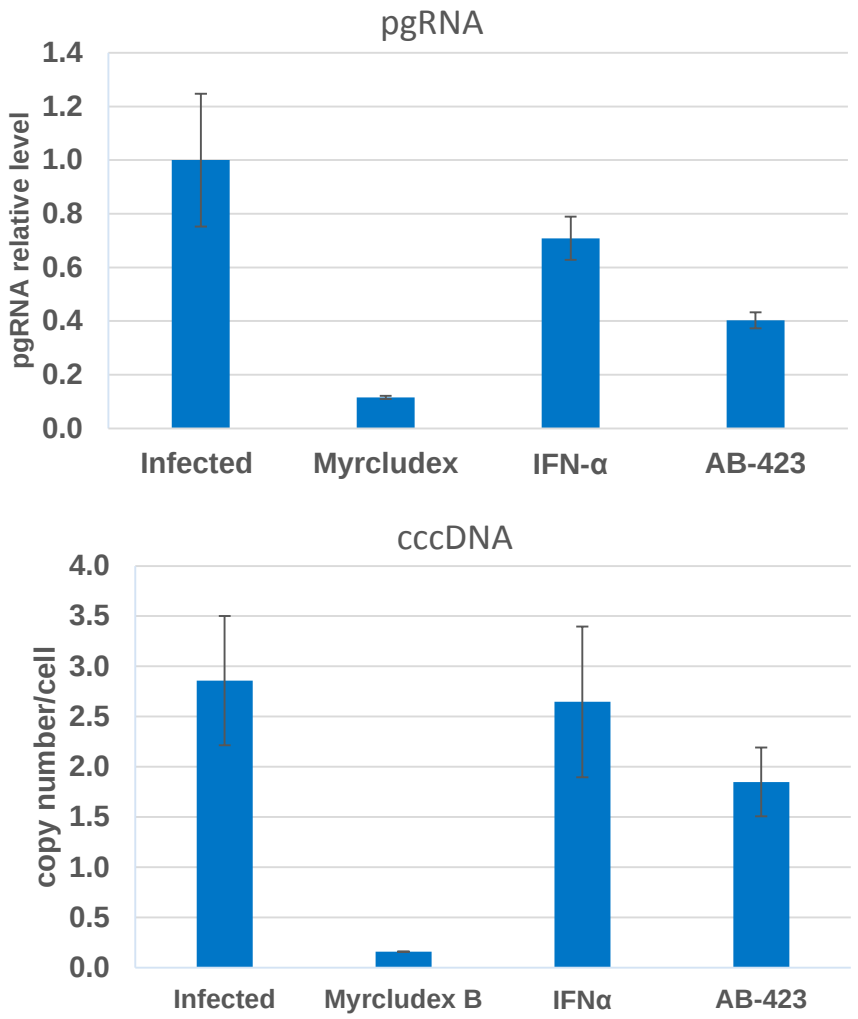
Virus	Family	Genome	EC_{50} (µM)	CC_{50} (µM)	Host Cell Line
Hepatitis C	Flaviviridae	(+) ssRNA	11.2	>30	Huh7
WNV	Flaviviridae	(+) ssRNA	>30	19	VERO
Dengue Virus	Flaviviridae	(+) ssRNA	>30	>30	Huh7
Rhinovirus (HRV 1A)	Picornaviridae	(+) ssRNA	7.18	>30	H1/HeLa
Influenza A Virus	Orthomyxoviridae	segmented (-) ssRNA	>30	>30	MDCK
RSV	Paramyxoviridae	non-segmented (-)ssRNA	19.2	>30	HEp2
Human Cytomegalovirus	Herpesviridae	dsDNA	>30	>30	MRC5
Herpes Simplex Virus	Herpesviridae	dsDNA	>30	>30	VERO
HIV	Retroviridae	ssRNA to DNA	>30	16.2	CEMSS

AB-423 Inhibits Viral Replication



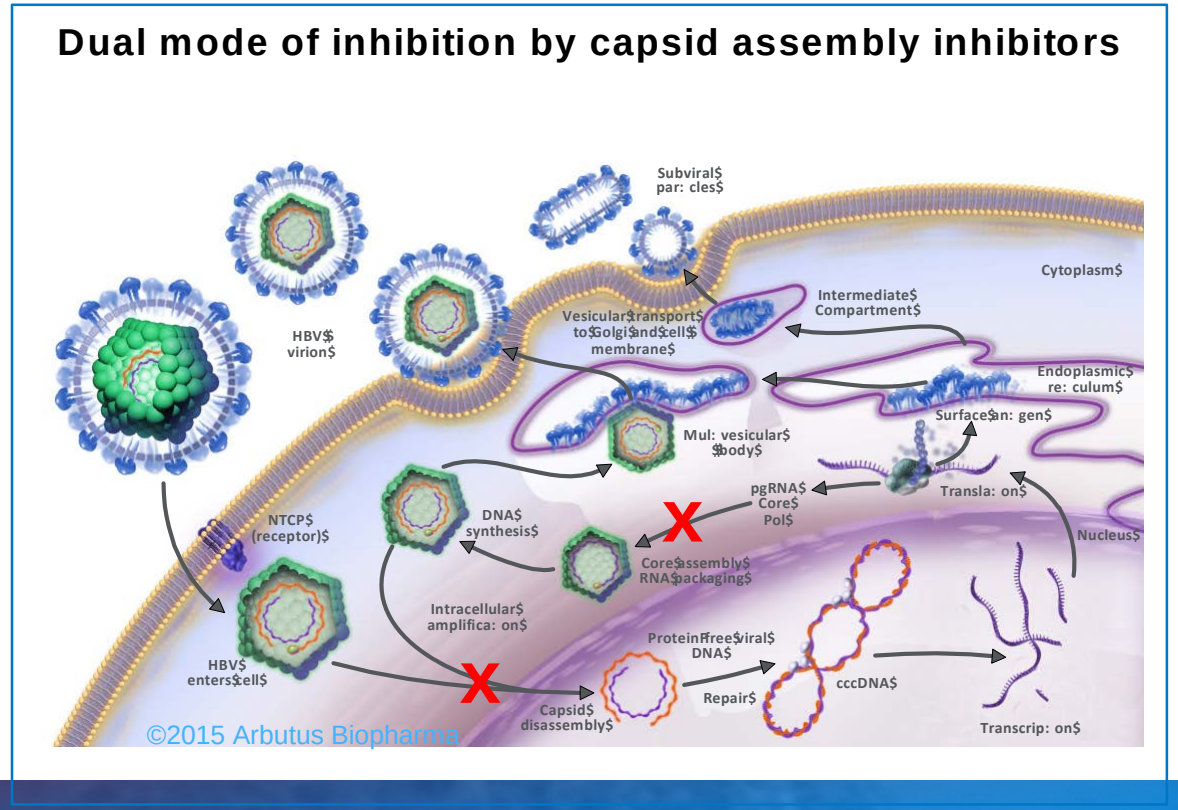
AB-423 Inhibits Conversion of Encapsidated rcDNA to cccDNA in an Infectious Virus System

- AB-423 inhibits cccDNA and pgRNA synthesis during *de novo* HBV infection of C3A^{hNTCP} cells
- AB-423 Inhibits cccDNA synthesis presumably *via* inhibition of the capsid uncoating step

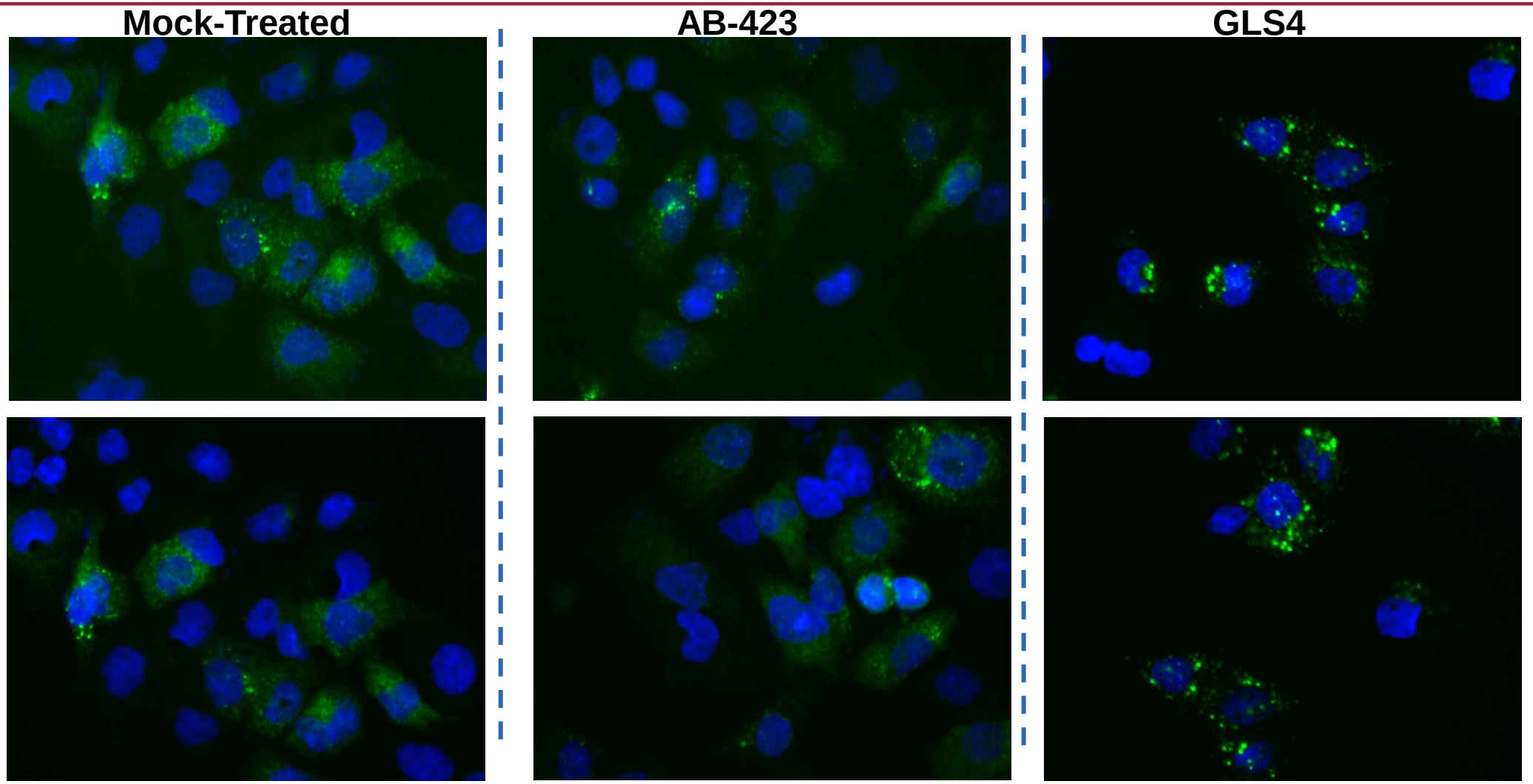


AB-423 Inhibits Conversion of Encapsidated rcDNA to cccDNA in an Infectious Virus System

- Data suggests AB-423 has a dual mode of inhibition:
  - Inhibits encapsidation of pgRNA during ongoing infection
  - Inhibits cccDNA synthesis presumably *via* inhibition of the capsid uncoating step



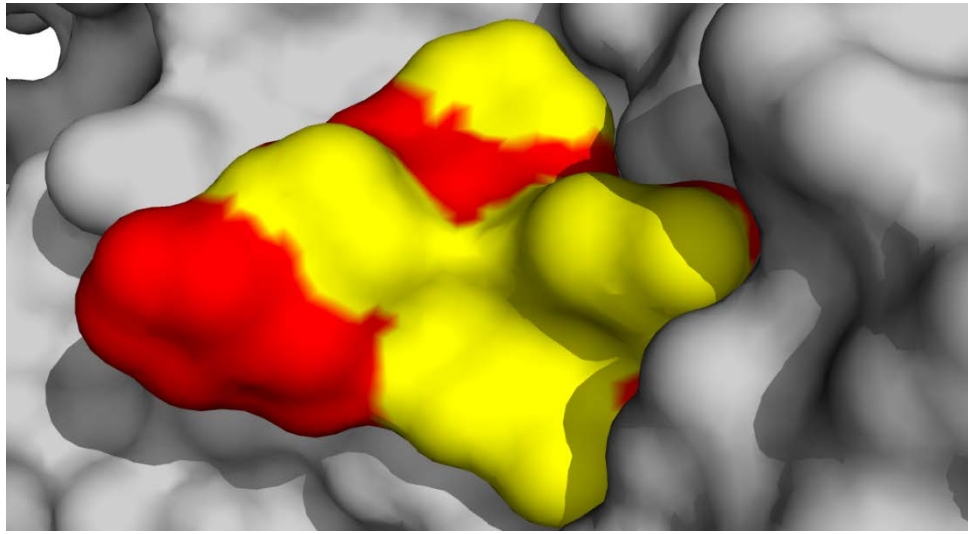
AB-423 Does Not Induce Core Aggregation in HBV-Replicating HepAD38 Cells



-5 day treatment, both compounds at 3 µM. DAPI=Nuclei, FITC=anti-Core Ab

Structural Insights into Binding of Core Protein Allosteric Modulators (CpAM)

- Two classes of CpAMs have been defined
  - Class I CpAMs induce non-capsid polymers
  - Class II CpAMs allows capsid formation devoid of pgRNA
- High resolution X-ray structures of capsid inhibitors bound to capsid protein have been published
- Class I and II core protein assembly modulators bind to the same site, the dimer:dimer interface, yet have different effects on HBV biology
- Molecule related to AB-423 binds in the same site



Overlay of a NVR-010–001-E2, a class I CpAM, (HAP, Yellow) and a novel Class II CpAM (Red) bound to CpY132A core protein. Bourne *et al* 2006; Katen *et al* 2013; Klumpp *et al* 2015; Qiu *et al* 2016; Cole, 2016; Arbutus Biopharma unpublished data

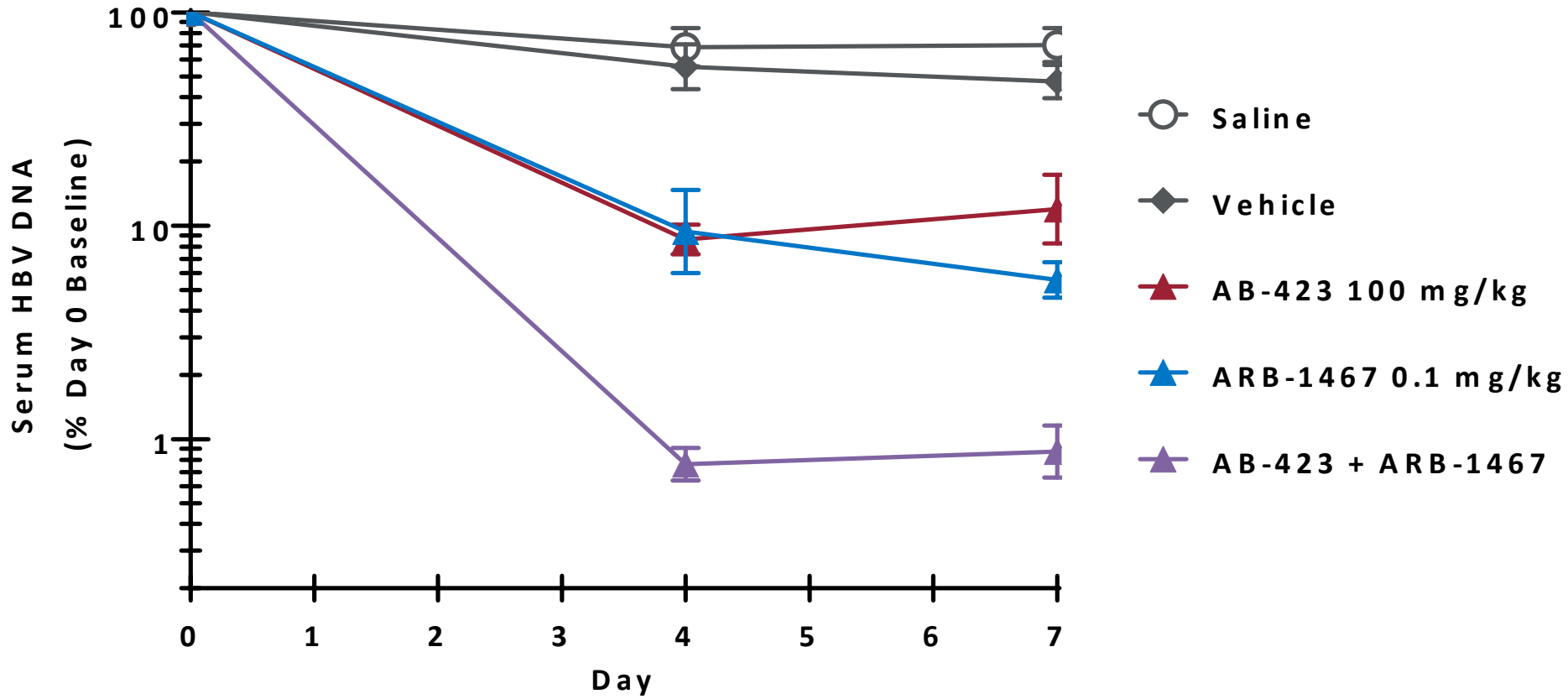
In vitro Data Indicates Potential for Combining AB-423 with Nucs, IFN, and RNAi agents

	Inhibitor B	Cell Culture Model	Conclusion*
pgRNA → rcDNA → cccDNA			
AB-423	ARB-1740 (*RNAi 2.0*)	HepBHAe82 (precore RNA/qRT-PCR)	Synergy
AB-423	ETV	HepBHAe82 (precore RNA/qRT-PCR)	Synergy
pgRNA → rcDNA			
AB-423	ARB-1467 (*RNAi 1.0)*	AML12-HBV10 (bDNA/rcDNA)	Additive
AB-423	ARB-1740 (*RNAi 2.0*)	AML12-HBV10 (bDNA/rcDNA)	Additive
AB-423	ETV	AML12-HBV10 (bDNA/rcDNA)	Additive
AB-423	TDF	HepDE19 (bDNA/rcDNA)	Additive
rcDNA and eAg			
AB-423	TAF	HBV infected PHH (HBV DNA/HBeAg)	Additive
AB-423	IFN	HBV infected PHH (HBV DNA/HBeAg)	Synergy

*MacSynergy II Analysis; Bliss Independence Model; Prichard and Shipman 1990. Antiviral Research, 14(4-5):181-205

- Combination of AB-423 with RNAi agents, Nucs, or IFN is supported by additive to synergistic antiviral activity in *in vitro* studies

Enhanced Activity for AB-423 in Combination with siRNA ARB-1467



- *In vivo* combination of AB-423 with RNAi agent 1467 in a HDI mouse is supportive with *in vitro* observed additive effects

Summary

- AB-423 is a potent, highly selective inhibitor of HBV replication.

- AB-423 showed dual mode of inhibition:
  - *inhibited encapsidation of pgRNA during ongoing infection*
  - *inhibited cccDNA synthesis presumably via inhibition of the capsid uncoating step*

- *In vitro* AB-423 showed:
  - *pan-genotypic activity*
  - *potent activity against HBV Nuc^R variants*
  - *additive/synergistic activity in combination with Nucs, IFN, and RNAi agents*
  - *no significant activity against unrelated viruses*

- Results indicate that HBV encapsidation inhibitors show significant distinctions in mechanism of antiviral activity from the Nucs

Acknowledgements

Arbutus Biopharma

- Nagraj Mani
- Andrew G. Cole
- Andrzej Ardzinski
- Andrea Cuconati
- Bruce D. Dorsey
- Steven Kultgen
- Amy Lee
- Rene Rijnbrand
- Nicholas Snead
- Holly Steuer
- Xiaohe Wang
- Michael J. Sofia

Baruch S. Blumberg Institute

- Fang Guo
- Ju-Tao Guo
- Qiong Zhao

Indiana University

- Dawei Cai
- Haitao Guo