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

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Disclosure Statement

Employees of Arbutus Biopharma:
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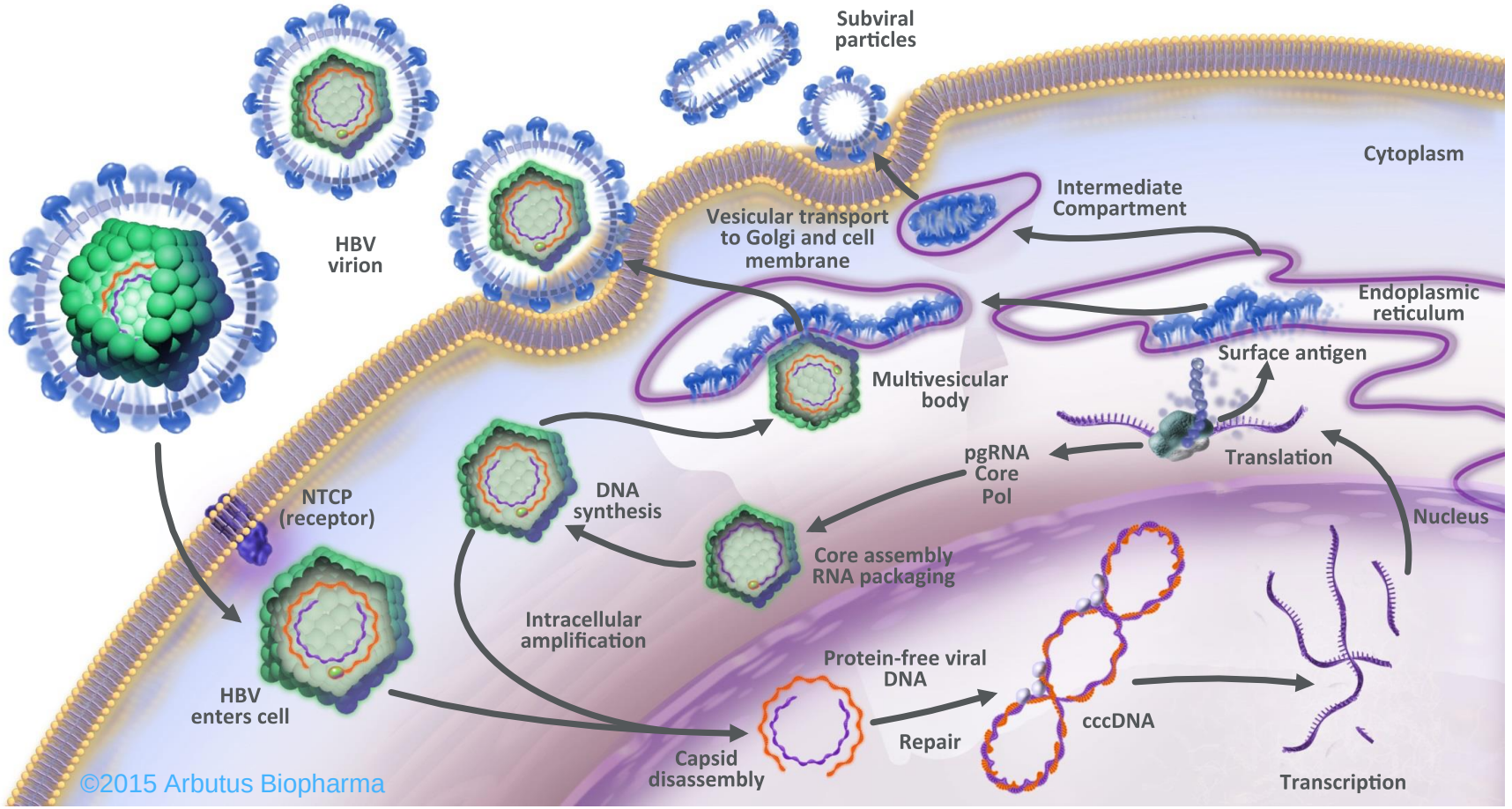
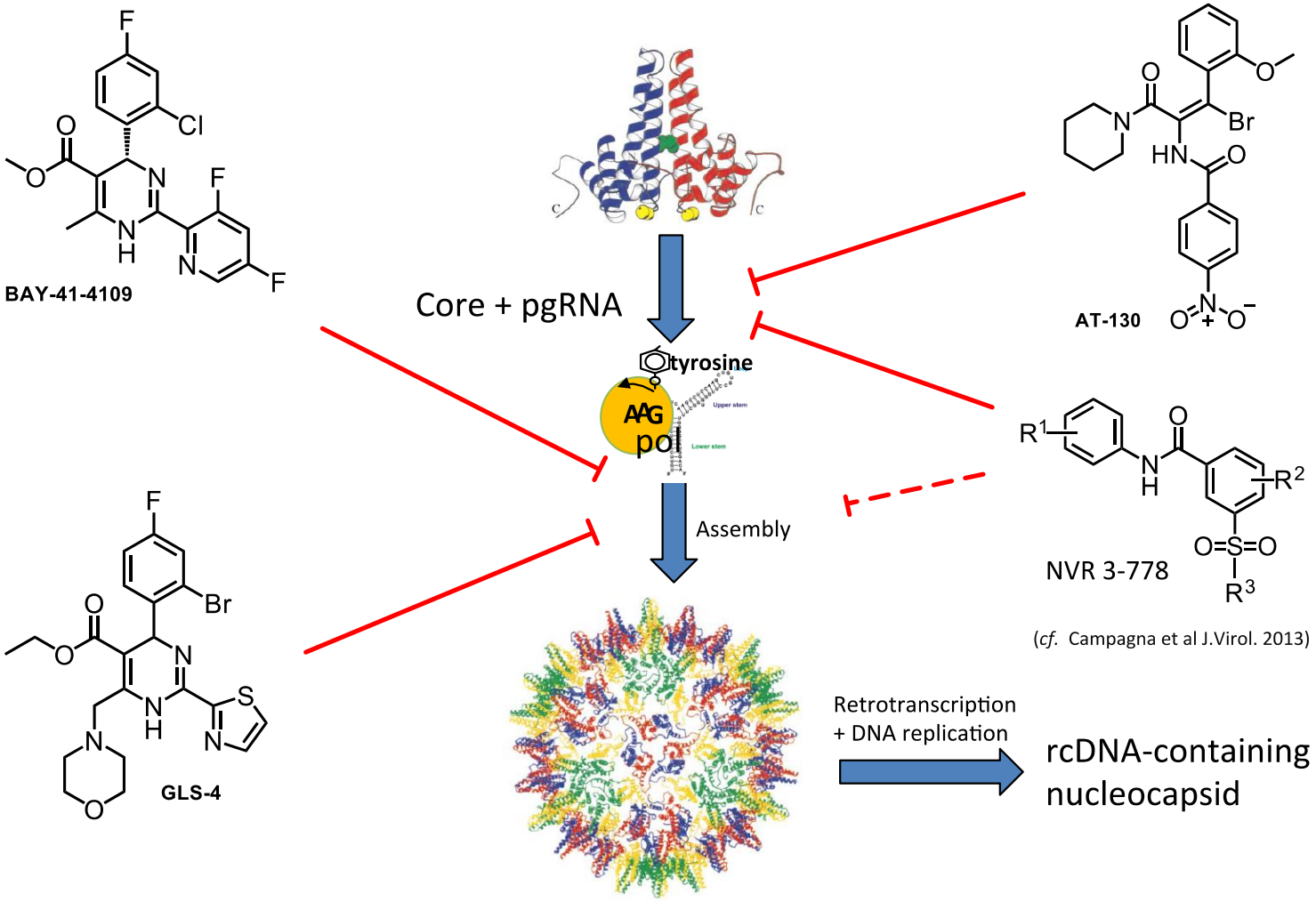
HBV Life Cycle



Capsid Assembly is a Validated Target

- Hepatitis B virus replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA) prior to rcDNA synthesis 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 novel 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



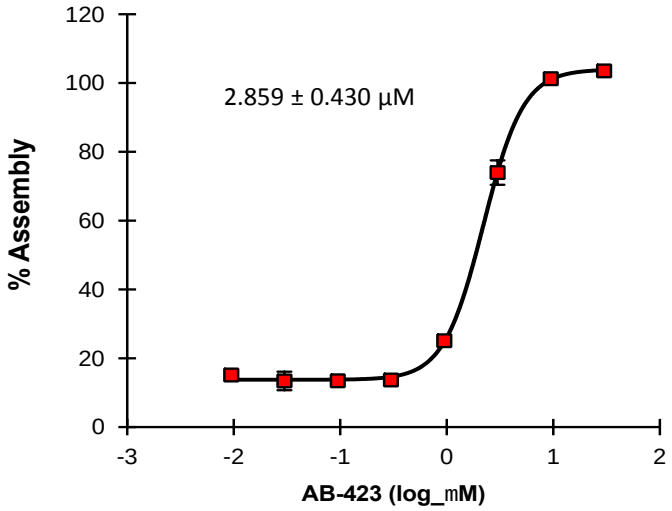
Hu et al., Ann. Rep. in Med. Chem. 2013

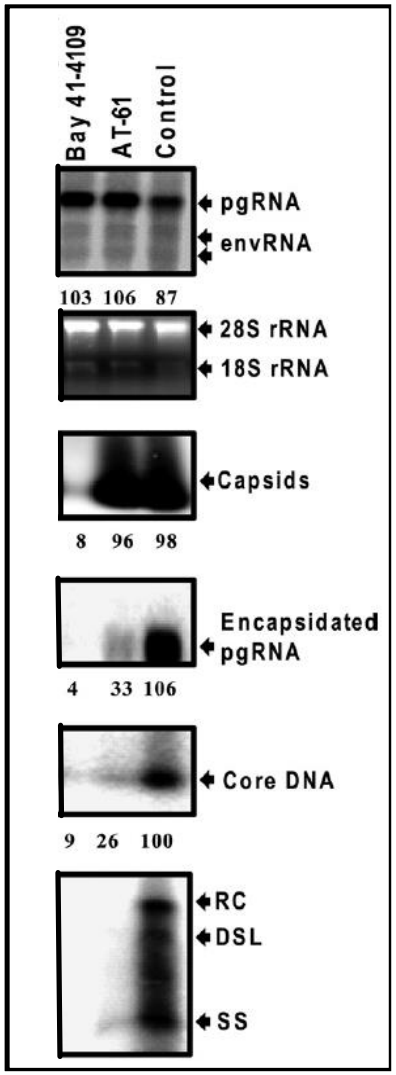
Winne et al, Mol.Cell 1999

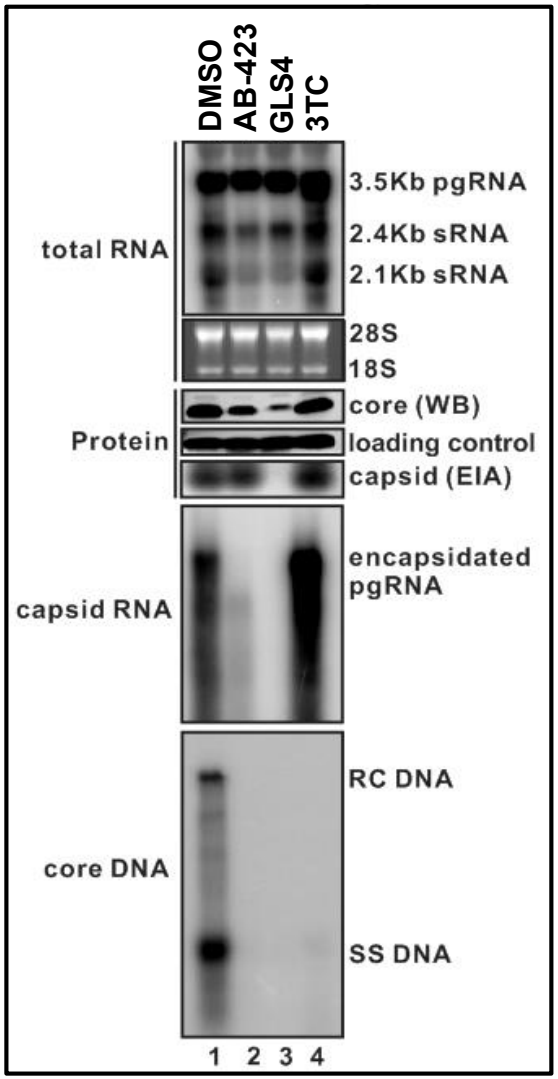
AB-423 Inhibits HBV pgRNA Encapsidation and Misdirects Capsid Assembly *In Vitro*

- In a biochemical capsid assembly assay, AB-423 misdirects capsid assembly
- AB-423 inhibits pgRNA encapsidation in an HBV cell culture model system

Capsid Assembly Biochemical Assay



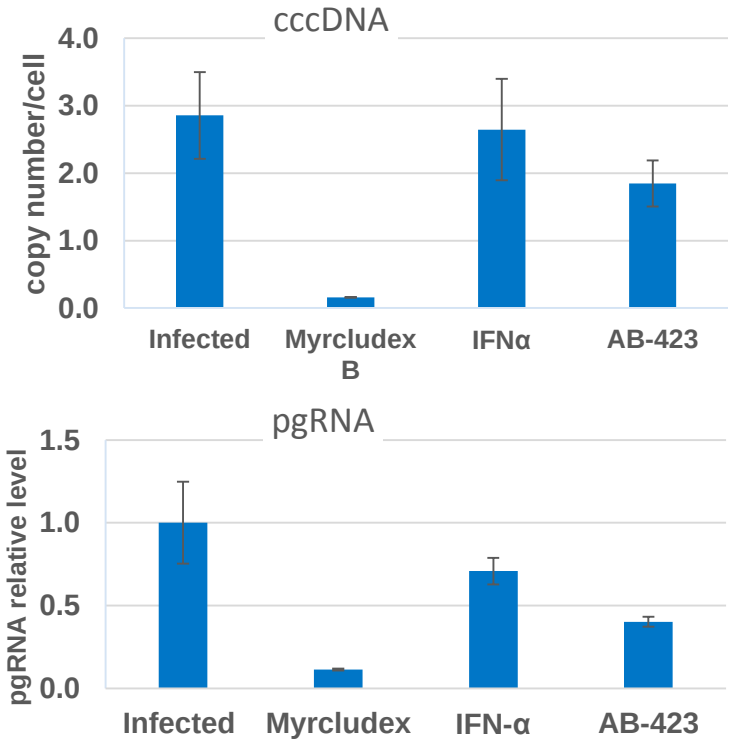




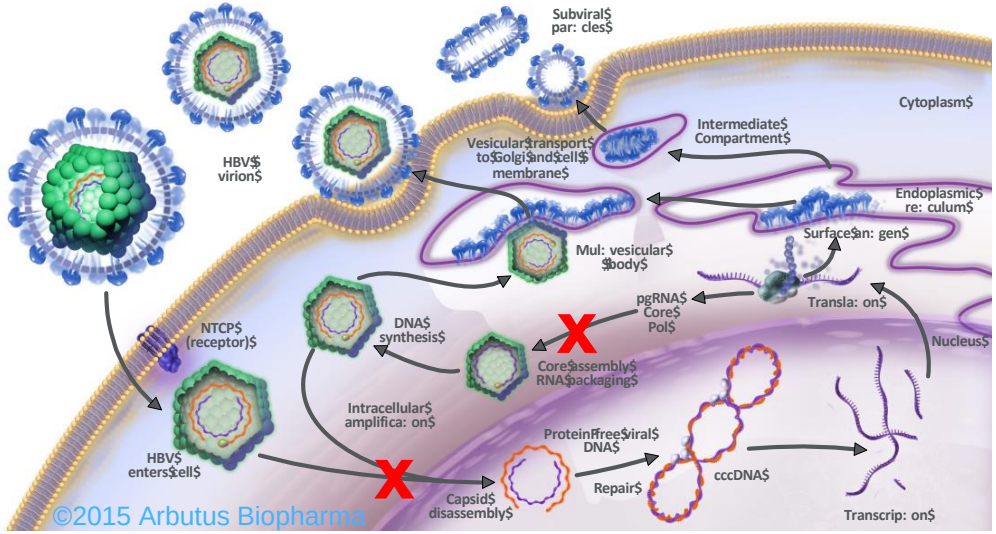
AB-423 Inhibits Conversion of Encapsidated rcDNA to cccDNA in an Infectious Virus System

- AB-423 inhibited cccDNA synthesis during *de novo* HBV infection of C3A^{hNTCP} cells

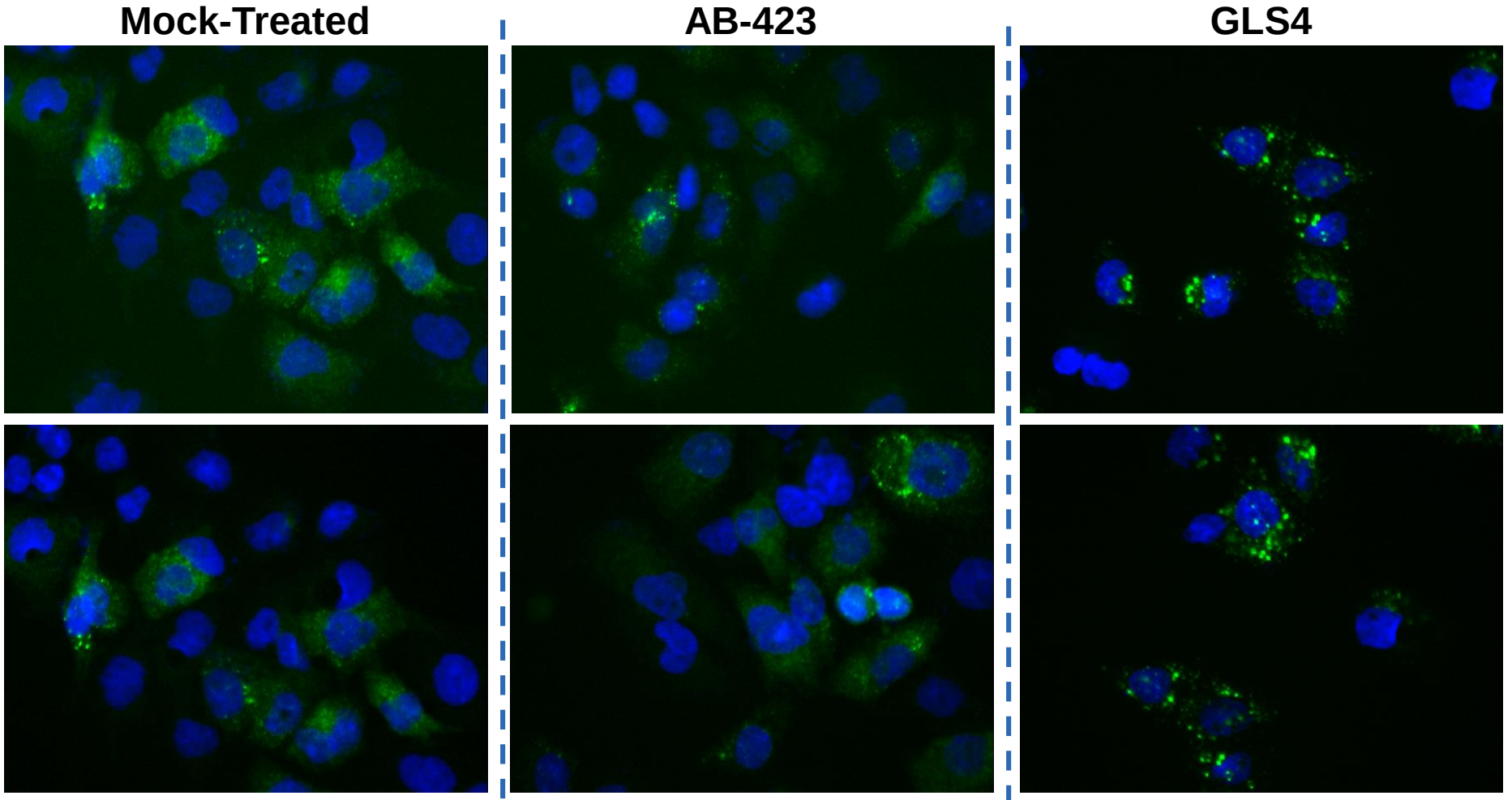
- Data suggests AB-423 has a dual mode of inhibition:
  - Inhibits encapsidation of pgRNA during ongoing infection
  - Inhibits cccDNA synthesis presumably *via* inhibition of the capsid uncoating step



Dual mode of inhibition by capsid assembly inhibitors



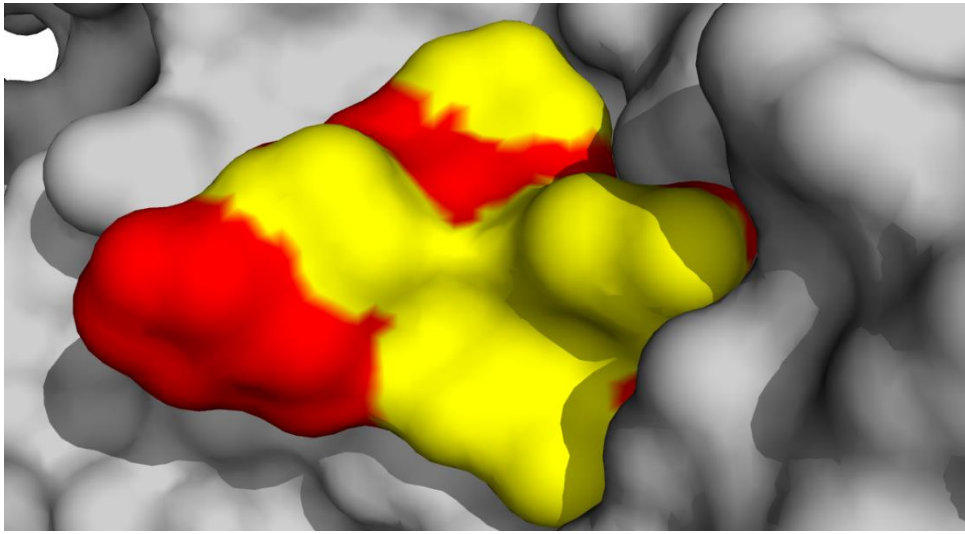
AB-423 Does Not Induce Core Aggregation in HBV-Replicating HepAD38 Cells



-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 HAPs (class I) bound to core 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



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

HAP: Heteroaryldihydropyrimidines

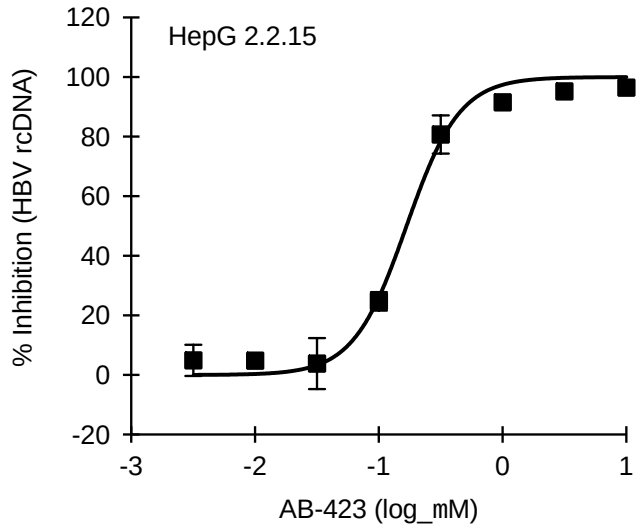
AB-423 is an Inhibitor of HBV Replication

- AB-423 is a potent inhibitor of HBV replicon in HBV cell-culture models

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

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

- Maintains activity across gt A-D (0.2-0.5 fold vs gt D)
- Maintains activity against nuc variants (1.7-2.2 fold shift vs wt gt D)
- No activity against heterologous RNA and DNA viruses



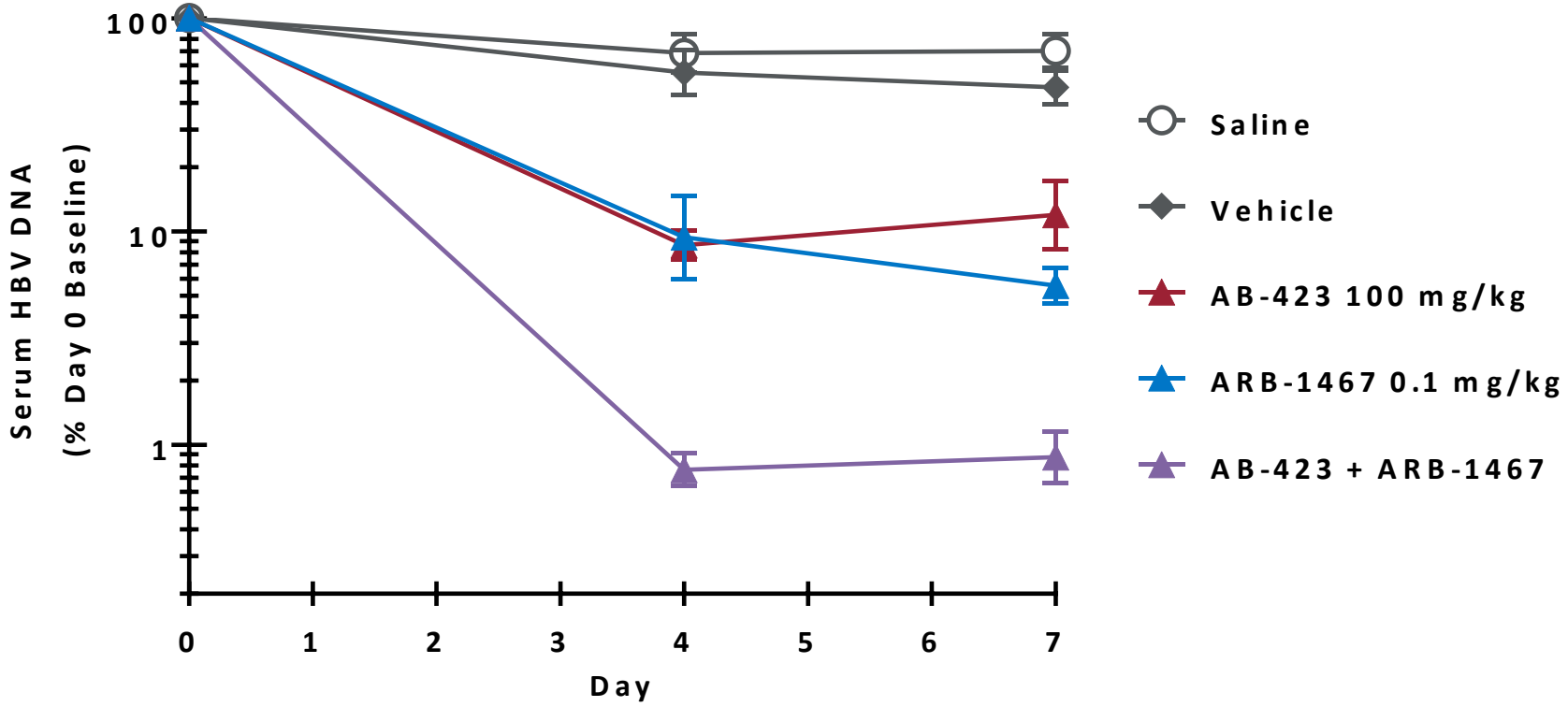
In vitro Data Indicates Potential for Combining AB-423 with Nucs and RNAi agents

Inhibitor A	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-1740 (*RNAi 2.0*)	AML12-HBV10 (bDNA/rcDNA)	Additive
AB-423	ARB-1467 (*RNAi 1.0*)	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

*MacSynergy II Analysis; Bliss Independence Model; Prichard and Shipman 1990. Antiviral Research, 14(4-5):181-205; ETV = entecavir; TDF = tenofovir disoproxil fumarate; TAF = tenofovir alafenamide

- Combination of AB-423 with RNAi agents and Nucs 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 through a block of pgRNA encapsidation.**
- ***In vitro* AB-423 showed:**
  - ***additive/synergistic activity in combination with Nucs and RNAi agents***
  - ***potent activity against HBV Nuc^R variants and pan-genotypic activity***
  - ***no significant activity against unrelated viruses***
- **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***
- **Results indicate that HBV encapsidation inhibitors show significant distinctions in mechanism of antiviral activity from the Nucs**

Acknowledgements

Arbutus Biopharma

- Nagraj Mani
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