Abstract #8

A Next Generation HBV Capsid Inhibitor, AB-506: In Vitro and In Vivo Antiviral Characterization

Nagraj Mani PhD
Arbutus Biopharma Inc.


Disclosure Statement: This work includes co-authors who are employees of Arbutus Biopharma
HBV Capsid Assembly

An attractive target for drug development

HBV capsid assembly pathway and examples of capsid inhibitors

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

cccDNA = covalently closed circular DNA; rcDNA = relaxed circular DNA; pgRNA = pregenomic RNA

**HBV capsid assembly pathway and examples of capsid inhibitors**

**Class I**
- Forms aberrant non-capsid polymers

**Class II**
- Forms empty capsid devoid of pgRNA/rcDNA

**Core + pgRNA**

**PP**

**AT-130**

**HAP**

**BAY-41-4109**

**RG7907**

**GLS-4**

**SBA**

**NVR 3-778**

**AB-423**

**AB-506**

**JNJ-379**

**ABI-H0731**

**rgDNA-containing nucleocapsid**

Retrotranscription + DNA replication

**HAP**: heteroarylidihydropyrimidines; **SBA**: sulfamoylbenzamides; **PP**: phenylpropenamides
AB-506 Is A Next Generation HBV Capsid Inhibitor

- AB-506 is our 2nd generation HBV capsid inhibitor from a novel chemical series
- Demonstrates potent inhibition of viral replication in different HBV cell culture models

<table>
<thead>
<tr>
<th>Compound</th>
<th>HepDE19 (rcDNA_bDNA) (μM)</th>
<th>HepBHAe82 (HBeAg AlphaLISA) (μM)</th>
<th>HepG 2.2.15 (HBV DNA qPCR) (μM)</th>
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<tbody>
<tr>
<td></td>
<td>EC₅₀</td>
<td>EC₉₀</td>
<td>CC₅₀</td>
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<tr>
<td>AB-506</td>
<td>0.07 ± 0.02</td>
<td>0.28 ± 0.10</td>
<td>&gt;25</td>
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</table>

- In an HBV infected primary human hepatocyte assay, AB-506 inhibits HBV replication with an EC₅₀ of 0.03 μM
- Maintains activity in the presence human serum with a modest ~6 fold increase in EC₅₀ in 40% human serum
- No cross-resistance with Nuc^{R} variants, consistent with its distinct mechanism of action
- Active against the most prevalent HBV genotypes (A-D) globally
- Demonstrates high degree of antiviral selectivity for HBV; no inhibition of HCV, WNV, RSV, IFA, HSV, HCMV, DENV, HRV
AB-506 Binds To Core Protein At The Dimer:Dimer Interface

Increases thermal stability of core protein; accelerates capsid assembly

- X-ray crystal structure of AB-506 with Cp-Y132A mutant solved (2.5Å)
- AB-506 binds at the dimer:dimer interface similar to other known Class I (HAP) and Class II (SBA) capsid inhibitors
- AB-506 binding increases thermal stability of WT core protein by 6 °C.
- In a biochemical capsid assembly assay, AB-506 accelerated capsid assembly
AB-506 Forms Empty Capsids Devoid of pgRNA or rcDNA

Mechanistic differentiation from nucleos/tide analogs (NA) and class I capsid inhibitors

- Mode of action studies conducted in AD38 cells
- Capsid formation maintained with AB-506 treatment
- AB-506 forms empty capsids devoid of pgRNA or rcDNA
- AB-506 MoA is consistent with a Class II inhibitor
- Distinct from GLS4, a Class I inhibitor and NA’s

GLS4 = 3 μM; Entecavir = 1 μM, AB-506 = 1 μM; SBA = 3 μM
**AB-506 Shows Potential For QD Dosing In Humans**

Pharmacokinetic studies in mouse, rat and dog

**Oral PK parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mice</th>
<th>Rats</th>
<th>Dogs</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1/2}$ (h)</td>
<td>2.6</td>
<td>4.3</td>
<td>11.4</td>
</tr>
<tr>
<td>$F$ (%)</td>
<td>~100</td>
<td>~100</td>
<td>~100</td>
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<tr>
<td>24 hr liver/plasma</td>
<td>3.0</td>
<td>3.5</td>
<td>NA</td>
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</table>

PK evaluation in multiple species shows favorable exposure and significant liver accumulation, supportive of QD dosing in humans

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**CD-1 mice**

- PO 10 mg/kg
- PO 30 mg/kg
- PO 100 mg/kg
- EC90

**SD rats**

- PO 10 mg/kg
- PO 30 mg/kg
- PO 100 mg/kg
- EC90

**Beagle dogs**

- PO 10 mg/kg
- EC90
AB-506 shows dose-responsive antiviral activity \textit{in vivo}

Antiviral activity in a mouse HDI model of HBV

The \textit{in vivo} antiviral activity was assessed in a hydrodynamic injection (HDI) HBV mouse model utilizing pHBV1.3 (Guidotti 1995). Test article was 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.
• 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 including HBV infected PHH
  - demonstrated pan-genotypic activity (A-D) and potency against Nuc\(^R\) variants;
    did not inhibit a panel of other viruses
  - 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 gen. capsid inhibitors

• Dosing performed in multiple species suggest QD potential and significant liver concentrations achieved

• AB-506 showed potent *in vivo* anti-viral activity in a HDI mouse model of HBV
  - *Even low-dose AB-506 substantially reduced liver HBV DNA*

• AB-506 is currently being evaluated for advancement into clinical development by mid 2018
# Acknowledgments

## Arbutus Team

<table>
<thead>
<tr>
<th>Nagraj Mani</th>
<th>Laurel Fu</th>
<th>Angela Miller</th>
</tr>
</thead>
<tbody>
<tr>
<td>Andrew G. Cole</td>
<td>Fang Guo</td>
<td>Chris Pasetka</td>
</tr>
<tr>
<td>Janet R. Phelps</td>
<td>Troy O. Harasym</td>
<td>Stephen P. Reid</td>
</tr>
<tr>
<td>Cory Abbott</td>
<td>Agnes Jarosz</td>
<td>Rene Rijnbrand</td>
</tr>
<tr>
<td>Andrzej Ardzinski</td>
<td>Salam Kadhim</td>
<td>Alexander Shapiro</td>
</tr>
<tr>
<td>Jeff Bechard</td>
<td>Steven G. Kultgen</td>
<td>Holly M. Steuer</td>
</tr>
<tr>
<td>Robbin Burns</td>
<td>Kaylyn Kwak</td>
<td>Kim Stever</td>
</tr>
<tr>
<td>Tim Chiu</td>
<td>Amy C.H. Lee</td>
<td>Sunny Tang</td>
</tr>
<tr>
<td>Andrea Cuconati</td>
<td>Alice H. Li</td>
<td>Xiaowei Teng</td>
</tr>
<tr>
<td>Bruce D. Dorsey</td>
<td>Sara Majeski</td>
<td>Xiaohe Wang</td>
</tr>
<tr>
<td>Ellen Evangelista</td>
<td>Kevin McClintock</td>
<td>Michael J. Sofia</td>
</tr>
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