HBV Capsid Inhibitors

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Chief Scientific Officer
Key to Therapeutic Success

REDUCE/SUPPRESS VIRAL DNA & ANTIGENS

- Viral Replication
- HBsAg
- cccDNA Formation/Function

RE-AWAKEN/BOOST IMMUNE RESPONSE

- Reduced HBsAg
- Immunotherapy

A combination approach to these key factors will drive cures
HBV Life Cycle

HBV enters cell
NTCP (receptor)

HBV virion

Vesicular transport to Golgi and cell membrane

Multivesicular body

Core assembly
RNA packaging

Capsid disassembly

DNA synthesis

Intracellular amplification

Subviral particles

Intermediate Compartment

Endoplasmic reticulum

Cytoplasm

Surface antigen

Nucleus

Transcription

cccDNA

Protein-free viral DNA

Repair

Transcription

Cytoplasm

Endoplasmic reticulum

Surface antigen

Nucleus

Transcription

cccDNA

Protein-free viral DNA

Repair

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Cytoplasm

Endoplasmic reticulum

Surface antigen

Nucleus

Transcription

cccDNA

Protein-free viral DNA

Repair

Transcription
Hepatitis B virus replication is strictly dependent upon capsid assembly around pregenomic RNA (pgRNA) prior to rcDNA and subsequent cccDNA synthesis.

Interfering with HBV capsid assembly has translated into antiviral activity in vitro and in vivo and constitutes a novel MOA over approved therapies.

Capsid assembly inhibitors show significant distinctions in mechanism of antiviral activity vs currently approved NUCs.

Two classes of capsid assembly inhibitors exist:
- Class I forms aberrant linear polymers
- Class II forms empty capsids

All capsid assembly inhibitors bind to the same core protein pocket.

Multiple capsid assembly inhibitor compounds in clinical development (Capsid Inhibitors, Capsid Assembly Inhibitors and Core Protein Allosteric Modulators (CpAMs) are the same).
HBV capsid assembly pathway

Class I and Class II Capsid Inhibitors

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

- **BAY-41-4109**
- **RG7907**
- **GLS-4**

**HAP**

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

- **AT-130**
- **AB-423**
- **AB-506**
- **JNJ-379**
- **ABI-H0731**

**Core + pgRNA**

**tyrosine**

**Mg pol**

**NVR 3-778**

**SBA**

**PP**

**AT-130**

**rcDNA-containing nucleocapsid**

**HAP**: heteroaryldihydropyrimidines; **SBA**: sulfamoylbenzamides; **PP**: phenylpropenamides
Two Known Classes of Capsid Assembly Inhibitors

Most Companies Focused on Class II

- Class I (Heteroaryldihydropyrimidines (HAP)) – Forms aberrant linear polymers of core protein
- Class II (Propenamides/Sulfonylbenzamides) – Form empty capsids
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

\[ IC_{50} : 2.9 \, \mu M \]

- AB-423 inhibits pgRNA encapsidation in an HBV cell culture model system
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
AB-423 is an Inhibitor of HBV Replication

<table>
<thead>
<tr>
<th>Assay</th>
<th>HepG 2.2.15</th>
<th>HepDE19</th>
<th>AML12-HBV10</th>
<th>HepBHAe82</th>
<th>PHH</th>
</tr>
</thead>
<tbody>
<tr>
<td>EC_{50} (µM)*</td>
<td>0.146±0.024</td>
<td>0.262±0.127</td>
<td>0.263±0.177</td>
<td>0.267±0.135</td>
<td>0.078±0.031</td>
</tr>
<tr>
<td>EC_{90} (µM)*</td>
<td>0.993±0.855</td>
<td>0.905±0.332</td>
<td>1.319±1.076</td>
<td>1.246±0.466</td>
<td>0.333±0.0235</td>
</tr>
<tr>
<td>CC_{50} (µM) #</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Assay</td>
<td>(rcDNA/qPCR) human hepatoma cell line</td>
<td>(rcDNA/bDNA) human hepatoma cell line</td>
<td>(rcDNA/bDNA) mouse hepatoma cell line</td>
<td>(eAg/ELISA) human hepatoma cell line</td>
<td>(virion DNA/qPCR) Primary human hepatocytes</td>
</tr>
</tbody>
</table>

* EC_{50}/EC_{90} ± SD
# Highest concentration tested
AB-423 has Pan Genotypic Activity

- Most tissue culture systems represent gt D

<table>
<thead>
<tr>
<th>Genotype</th>
<th>AB-423 EC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>0.057</td>
</tr>
<tr>
<td>A-2</td>
<td>0.089</td>
</tr>
<tr>
<td>B-1</td>
<td>0.039</td>
</tr>
<tr>
<td>B-2</td>
<td>0.091</td>
</tr>
<tr>
<td>C-1</td>
<td>0.052</td>
</tr>
<tr>
<td>C-2</td>
<td>0.055</td>
</tr>
<tr>
<td>D</td>
<td>0.195</td>
</tr>
</tbody>
</table>

- 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<sup>R</sup> Variants

<table>
<thead>
<tr>
<th>HBV Variant</th>
<th>AB-423 EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>ETV EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
<th>LAM EC&lt;sub&gt;50&lt;/sub&gt; (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rtM204I</td>
<td>0.192</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rtM204I+V173L</td>
<td>0.151</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rtM204I+S202G</td>
<td>0.190</td>
<td>10.7</td>
<td>ND</td>
</tr>
<tr>
<td>rtM204V+L180M</td>
<td>0.175</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rtM204I+S202G+M250V</td>
<td>0.235</td>
<td>9.042</td>
<td>ND</td>
</tr>
<tr>
<td>U95551 (WT, GtD)</td>
<td>0.105</td>
<td>0.002</td>
<td>0.03</td>
</tr>
<tr>
<td>rtM204I</td>
<td>0.192</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
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<td>0.151</td>
<td>ND</td>
<td>&gt;100</td>
</tr>
<tr>
<td>rtM204I+S202G</td>
<td>0.190</td>
<td>10.7</td>
<td>ND</td>
</tr>
</tbody>
</table>

- No cross-resistance with Nuc<sup>R</sup> variants. Consistent with their distinct mechanisms of action.
AB-423 is a Selective Inhibitor of HBV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Family</th>
<th>Genome</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>CC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>Host Cell Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis C</td>
<td>Flaviviridae</td>
<td>(+) ssRNA</td>
<td>11.2</td>
<td>&gt;30</td>
<td>Huh7</td>
</tr>
<tr>
<td>WNV</td>
<td>Flaviviridae</td>
<td>(+) ssRNA</td>
<td>&gt;30</td>
<td>19</td>
<td>VERO</td>
</tr>
<tr>
<td>Dengue Virus</td>
<td>Flaviviridae</td>
<td>(+) ssRNA</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>Huh7</td>
</tr>
<tr>
<td>Rhinovirus (HRV 1A)</td>
<td>Picornaviridae</td>
<td>(+) ssRNA</td>
<td>7.18</td>
<td>&gt;30</td>
<td>H1/HeLa</td>
</tr>
<tr>
<td>Influenza A Virus</td>
<td>Orthomyxoviridae</td>
<td>segmented (-) ssRNA</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>MDCK</td>
</tr>
<tr>
<td>RSV</td>
<td>Paramyxoviridae</td>
<td>non-segmented (-)ssRNA</td>
<td>19.2</td>
<td>&gt;30</td>
<td>HEP2</td>
</tr>
<tr>
<td>Human Cytomegalovirus</td>
<td>Herpesviridae</td>
<td>dsDNA</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>MRC5</td>
</tr>
<tr>
<td>Herpes Simplex Virus</td>
<td>Herpesviridae</td>
<td>dsDNA</td>
<td>&gt;30</td>
<td>&gt;30</td>
<td>VERO</td>
</tr>
<tr>
<td>HIV</td>
<td>Retroviridae</td>
<td>ssRNA to DNA</td>
<td>&gt;30</td>
<td>16.2</td>
<td>CEMSS</td>
</tr>
</tbody>
</table>
AB-423 Inhibits Viral Replication

1. HBV virion enters the cell via the NTCP receptor.
2. HBV enters the cell.
3. DNA synthesis occurs.
4. Intracellular amplification takes place.
5. Vesicular transport to Golgi and cell membrane.
6. Multivesicular body formation.
7. Core assembly and RNA packaging.
8. Protein-free viral DNA synthesis.
9. Capsid disassembly.
10. Transcription of cccDNA.
11. Repair of DNA.
12. Protein-free viral DNA.
13. Subviral particles.
15. Intermediate compartment.
17. Nucleus.
18. Endoplasmic reticulum.
20. Core Pol.
21. pgRNA Core Pol.
22. Multivesicular body.
23. Subviral particles.
24. Protein-free viral DNA.
Capsid Inhibitors Have Multiple Modes of Action

- AB-423 inhibited cccDNA synthesis during *de novo* HBV infection of C3A<sup>hNTCP</sup> cells

- Data suggests AB-423 has multiple modes of inhibition:
  - Inhibits encapsidation of pgRNA during ongoing infection
  - Inhibits cccDNA synthesis presumably *via* inhibition of the capsid uncoating step:
    - Likely relevant for cccDNA recycling during ongoing infection and *de novo* infection of new hepatocytes
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
**In vitro** Data Indicates Potential for Combining AB-423 with Nucs, IFN, and RNAi agents

<table>
<thead>
<tr>
<th>Inhibitor B</th>
<th>Cell Culture Model</th>
<th>Conclusion*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-423</td>
<td>HepBHAe82 (precore RNA/qRT-PCR)</td>
<td>Synergy</td>
</tr>
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<td>Synergy</td>
</tr>
<tr>
<td>AB-423</td>
<td>AML12-HBV10 (bDNA/rcDNA)</td>
<td>Additive</td>
</tr>
<tr>
<td>AB-423</td>
<td>AML12-HBV10 (bDNA/rcDNA)</td>
<td>Additive</td>
</tr>
<tr>
<td>AB-423</td>
<td>AML12-HBV10 (bDNA/rcDNA)</td>
<td>Additive</td>
</tr>
<tr>
<td>AB-423</td>
<td>HepDE19 (bDNA/rcDNA)</td>
<td>Additive</td>
</tr>
<tr>
<td>AB-423</td>
<td>HBV infected PHH (HBV DNA/HBeAg)</td>
<td>Additive</td>
</tr>
<tr>
<td>AB-423</td>
<td>HBV infected PHH (HBV DNA/HBeAg)</td>
<td>Synergy</td>
</tr>
</tbody>
</table>

*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
AB-506
A Second Generation Capsid Inhibitor

<table>
<thead>
<tr>
<th>Compound</th>
<th>HepDE19 (rcDNA_bDNA)</th>
<th>HepBHAe82 (HBeAg AlphaLISA)</th>
<th>HepG 2.2.15 (HBV DNA qPCR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$</td>
<td>EC$_{90}$</td>
<td>CC$_{50}$</td>
</tr>
<tr>
<td>AB-506 (µM)</td>
<td>0.07</td>
<td>0.27</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

- AB-506 is a novel Capsid Inhibitor demonstrating potent cellular activity in multiple cell culture models of HBV

- In a biochemical assay, AB-506 demonstrates acceleration of capsid assembly
AB-506

Binds at the HBV Core Protein Dimer:Dimer Interface

- X-ray crystal structure of AB-506 with CpY132A mutant solved at 2.25 Å
- Compound binds at the dimer:dimer interface similar to other known Class I (HAP) and Class II (SBA) Capsid Inhibitors
AB-506

Mechanism of Action Studies

- Mode of action studies conducted in HepAD38 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 (HAP) inhibitor
AB-506

Potential Best in Class Profile, Superior Potency and PK Relative to AB-423

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

### Oral PK parameters

<table>
<thead>
<tr>
<th></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>
</tr>
<tr>
<td>24 hr liver/plasma</td>
<td>3.0</td>
<td>3.5</td>
<td>NA</td>
</tr>
</tbody>
</table>
**AB-506**

**Potential Best in Class Profile, Superior Potency and PK Relative to AB-423**

- Significantly more potent than AB-423 *in vitro*. Improved PK supports predicted QD dosing in humans with a reduced pill burden

### In Vitro Potency

<table>
<thead>
<tr>
<th>Cmpd</th>
<th>HepDE19 rcDNA, bDNA (µM)</th>
<th>HepBHAe82 HBeAg ELISA (µM)</th>
<th>HepG 2.2.15 HBV DNA qPCR (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC$_{50}$</td>
<td>EC$_{90}$</td>
<td>CC$_{50}$</td>
</tr>
<tr>
<td>AB-423</td>
<td>0.26</td>
<td>0.91</td>
<td>&gt;10</td>
</tr>
<tr>
<td>AB-506</td>
<td>0.07</td>
<td>0.27</td>
<td>&gt;25</td>
</tr>
</tbody>
</table>

### In Vivo PK (@10/2 mpk p.o./i.v.)

<table>
<thead>
<tr>
<th></th>
<th>(Mouse / rat / dog)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma Exposure, AUC$_{inf}$ (h*ng/mL)</td>
</tr>
<tr>
<td></td>
<td>2,238</td>
</tr>
<tr>
<td></td>
<td>9,867</td>
</tr>
</tbody>
</table>

- Greater Potency
- Greater Plasma Exposure
- Higher Plasma Concentration
- Reduced Clearance
AB-506

In vivo antiviral activity in a mouse HDI model of HBV

- AB-506 Demonstrates a dose dependent (up to 3 log) reduction in HBV DNA in a mouse HDI model of HBV

NOD-SCID ♀ mice ~6-7 weeks old

Terminate and harvest liver

pHBV1.3 plasmid DNA
AB-506

Potential Best in Class Profile, Superior Potency and PK Relative to AB-423

- AB-506 is significantly more potent than AB-423 in vitro

<table>
<thead>
<tr>
<th>Compound</th>
<th>Potency</th>
<th>HepDE19 (rcDNA_bDNA)(μM)</th>
<th>HepBHAe82 (HBeAg ELISA)(μM)</th>
<th>HepG 2.2.15 (HBV DNA qPCR)(μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-423</td>
<td>EC₅₀/EC₉₀/CC₅₀</td>
<td>0.26/0.91/&gt;10</td>
<td>0.27/1.25/&gt;10</td>
<td>0.15/1.0/&gt;10</td>
</tr>
<tr>
<td>AB-506</td>
<td>EC₅₀/EC₉₀/CC₅₀</td>
<td>0.07/0.27/&gt;25</td>
<td>0.04/0.20/&gt;25</td>
<td>0.04/ND/&gt;10</td>
</tr>
</tbody>
</table>

- AB-506 is significantly more potent than AB-423 in vivo, with better liver HBV DNA reductions than ETV
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<sup>R</sup> variants
- additive/synergistic activity in combination with Nucs, IFN, and RNAi agents
- no significant activity against unrelated viruses

AB-506 has greater potency and improved PK properties vs. AB-423

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