Preclinical antiviral drug combination studies utilizing novel orally bioavailable agents for chronic hepatitis B infection: AB-506, a next generation HBV capsid inhibitor, and AB-452, an HBV RNA destabilizer

Rene Rijnbrand
Arbutus Biopharma Inc.
Antivirals: Targeting HBV and Beyond
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>257M people are chronically infected with HBV, globally.

~900k people die every year as a consequence despite the availability of effective vaccines and antivirals.

The Hepatitis B Virus

Genome Structure of HBV

- 4 Promoter elements
- 2 enhancer elements
- 10 transcription start sites

5 mRNAs:
- Pregenomic/core/pol (3.5 kb)
- Precore (3.5 kb)
- PreS1 (2.4 kb)
- PreS2/S (2.1 kb)
- X (0.7 kb)

Source: Gerlich, W. 2013. Virology Journal, 10:239
Keys to Therapeutic Success

Therapeutic success will combine drugs with complementary MOAs.
Key to Therapeutic Success: Combining Agents with Different MOA

HBV RNA destabilizers: AB-452, siRNA: ARB-1467, AB-729

Capsid inhibitors: AB-423, AB-506
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

HAP: heteroaryldihydropyrimidines; SBA: sulfamoylbenzamides; PP: phenylpropenamides

cccDNA = covalently closed circular DNA; rcDNA = relaxed circular DNA; pgRNA = pregenomic RNA
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 showing EC50, EC90, CC50 values for AB-506 and other compounds](image)

- In an HBV infected primary human hepatocyte assay, AB-506 inhibits HBV replication with an EC50 of 32 nM
- Maintains activity in the presence human serum with a modest ~6 fold increase in EC50 in 40% human serum
- No cross-resistance with NucR variants, consistent with its distinct mechanism of action
- Active against HBV genotypes A-H
- Demonstrates high degree of antiviral selectivity for HBV; no inhibition of HCV, WNV, RSV, IFA, HSV, HCMV, DENV, HRV
AB-506: A Next Gen HBV Capsid Inhibitor

Potent inhibitor of HBV replication in vitro

- AB-506 forms capsids devoid of pgRNA
- Inhibits formation of rcDNA

Capsid inhibitors bind at the dimer:dimer interface of the core protein and induce the formation of empty capsid particles

Dose Dependent Reduction in serum HBV DNA in an HDI mouse model of HBV

[Graph showing dose-dependent reduction in serum HBV DNA]
AB-452: A Potent HBV RNA Destabilizer
Novel small molecule HBV RNA Destabilizer

**AB-452 In vitro Potency**

In HepG2.2.15 cells

<table>
<thead>
<tr>
<th>Compound Conc. (uM)</th>
<th>% HBV production Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x10^-5</td>
<td>0.0001</td>
</tr>
<tr>
<td>0.001</td>
<td>0.01</td>
</tr>
<tr>
<td>0.1</td>
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</table>

Virus production
0.2 nM

<table>
<thead>
<tr>
<th>PHH</th>
<th>sAg</th>
<th>EC_{50}, nM</th>
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<tbody>
<tr>
<td></td>
<td>eAg</td>
<td>8.7</td>
</tr>
<tr>
<td>HepG2/NTCP</td>
<td>sAg</td>
<td>8.7</td>
</tr>
<tr>
<td></td>
<td>eAg</td>
<td>3.6</td>
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</table>

Genotype*

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EC_{50}, nM</th>
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<tbody>
<tr>
<td>A</td>
<td>1.3</td>
</tr>
<tr>
<td>B</td>
<td>1.8</td>
</tr>
<tr>
<td>C</td>
<td>2.0</td>
</tr>
<tr>
<td>D</td>
<td>0.8</td>
</tr>
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</table>

Human serum effect
2x

- AB-452 is a potent, highly selective small molecule inhibitor of HBV replication through destabilization of HBV RNA
- *In vitro* AB-452 showed: drops in viral RNA, s/e/c Ag, and virion production
  - Pan-genotypic activity
  - No cross-resistance with Nuc^R variants
  - Highly degree of antiviral selectivity for HBV

Human serum effect
Multiple Stages of HBV Life Cycle Affected by AB-452

- HBV RNAs destabilized by AB-452:
  - viral gene expressions
  - DNA replication
  - virion assembly
AB-452: A Potent HBV RNA Destabilizer

Novel small molecule HBV RNA Destabilizer

- BID PO dosing resulted in up to 1.4 log10 serum HBsAg reduction. Correlated with liver HBV RNA levels.

- AB-452 significantly inhibited HBV replication and reduced viral RNA and antigens in an immunocompetent AAV mouse model.
Evaluation of the Effect of Multiple Compounds on HBV: In Vitro Synergy Studies

96-well plate containing cells infected by HBV or expressing HBV reporter

Test activity of the 2 compounds together

Add concentration range for 1st compound
Add concentration range for 2nd compound

Outcomes:
- Compounds work against each other: Antagonism
- Compounds don’t interfere with each other: Additive
- Compounds enhance each other: Synergy
Combination of AB-506 and AB-452 With NAs and siRNA
Molecules are mechanistically compatible
**In Vitro Combination Studies: Summary**

Molecules are mechanistically compatible

<table>
<thead>
<tr>
<th>HBV Inhibitor</th>
<th>ETV</th>
<th>TDF</th>
<th>TAF</th>
<th>ARB-1467</th>
<th>AB-506</th>
</tr>
</thead>
<tbody>
<tr>
<td>AB-506</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Next Gen Capsid Inhibitor*</td>
<td>Additive</td>
<td>Additive</td>
<td>Moderate Synergy</td>
<td>Additive</td>
<td>NA</td>
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<tr>
<td>AB-452</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>HBV RNA Destabilizer**</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td>sAg</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>Minor Synergy</td>
<td>ND</td>
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<td>HBV DNA</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate Synergy</td>
<td></td>
<td></td>
<td>Additive</td>
<td>ND</td>
<td>Additive</td>
</tr>
</tbody>
</table>

- *HepDE19 HBV cell culture model with rcDNA quantitation
- **HepG2.2.15 HBV cell culture model with HBV DNA and HBsAg quantitation
Evaluation of the Effect of Multiple Compounds on HBV: In Vivo Evaluation

1. Administer HBV or reporter infected mouse
2. Outcomes:
   - Compounds work against each other: antagonism
   - Compounds don’t interfere with each other: additive or synergy

- Fewer test conditions can be examined in animals than in cell culture
- Prior dose selection is critical; mono-therapy arms are run at sub-optimal dose levels so that potential additive effect of combination therapy can be detected
**In Vivo Dual and Triple Combination of AB-506, AB-452 and TDF**

HDt Mouse Model of HBV: Serum HBV DNA and HBsAg Reductions

- Dual combinations of AB-506 + AB-452, AB-506 + TDF, and AB-452 + TDF showed strong antiviral activity with mean 1.4, 1.9 and 2.2 log_{10} reductions in serum HBV DNA vs the vehicle control, respectively.
- Triple combination effected larger serum HBV DNA reduction of 2.8 log_{10} vs the vehicle control.
- As expected, serum HBsAg reductions observed only in AB-452 groups.
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Once-Daily Oral Dose × 7 Days
Mean (n=7-8) ± SEM
Open symbol indicates close to LLOQ
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Once-Daily Oral Dose × 7 Days
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Open symbol indicates close to LLOQ.
In vivo Dual and Triple Combination of AB-506, AB-452 and TDF HDI Mouse Model of HBV: Liver HBV DNA and HBsAg Reductions

- Liver HBV DNA reductions reflect serum HBV DNA reductions
- AB-506 showed greater effect on liver HBV DNA reduction than TDF
- Only AB-452 containing groups showed liver HBsAg reductions
Summary

• Key to therapeutic success will involve combination of different MoA agents
  • Reduce/Suppress Viral DNA and Antigens
  • Reawaken/Boost host immune responses

• Agents with novel MoA undergoing clinical evaluation; more in preclinical stages
  • eg: Capsid Inhibitors, HBV RNA Destabilizers, RNAi Agents, NA, others

• *In vitro* and *in vivo* antiviral evaluations of Capsid Inhibitor AB-506, RNA Destabilizer AB-452, siRNA, and NA agents show favorable additive to synergistic effects in combination
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