Blockage of HBV Virus Replication and Inhibition of cccDNA Establishment by Core Protein Allosteric Modifiers (CpAMs)

Q. Huaoy, Yichao Zang, Alex Mercier, Emily Connolly, G. Renuka Kumar, David C., Cathal Mahon, Peter Cherney, Lida S. Zhao, Zhong Sun, Glynnis Church, Catherine Hahn, Lisa D. Arnold, Wham Tumre, Laping Li, Simon Hayter, Uh Lopatin and Richard Colombo
Assembly Biosciences, Inc.; San Francisco, CA, and Bloomington, IN, United States

Background
Approximately 240 million people worldwide are chronically infected with Hepatitis B virus (3% of the global population) and are at risk of developing chronic liver diseases, such as hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Currently approved treatments for chronic HBV include interferons (IFN) and nucleos(t)ide analogs (NAs), which are associated with high rates of therapy resistance and significant side effects. The development of new therapies appears to be their limited effect on cccDNA formation. Hence, new classes of anti-HBV molecules are needed to significantly improve cure rates in chronic HBV patients. HBV core protein (C) is involved in multiple steps of the HBV life cycle, including the formation, amplification and maintenance of cccDNA. A novel class of direct acting HBV antivirals, Core Protein Allosteric Modifiers (CpAMs), have been discovered to target HBV core protein and reduce HBV replication and cccDNA levels in Huh7 hepatocellular carcinoma cells. The mechanism of action of a series of proprietary CpAMs using a variety of predictive assays.

Materials and Methods

Inhibitors: Assembly Biosciences: CpAMs were synthesized at Acacia Sciences. GLS4 (CpAM) was synthesized by published procedures. 2'ETV (NA) was purchased from ACH BioMedicine.

Cell culture and compound treatment: Huh7 cells were transfected with plasmids expressing HBV constructs in 24 well plates and treated with compounds. Supernatants and cells were harvested at 5 days post transfection. Primary Human Hepatocytes (PHH; Lonza) were infected with HBV from Huh7 with ETV at 10x and 20x and treated with ETV at the indicated time points and concentrations. Supernatants and cells were harvested at indicated time points.

Materials profiling of CpAMs: HepG2 cells were treated with inhibitors and infected simultaneously. Cells were trypsinized 3 days later and harvested 6 days post-infection. HBV total RNA was isolated, pgRNA, Cp, cap, and capped-associated core DNA were analyzed by Northern blot, Western blot, Enzyme Immunoassay (EIA), and Southern blot, respectively, as previously described.

HBeAg quantifying assay: Huh7 cells were transfected with a plasmid expressing the complete HBeAg coding sequence under the control of a Tet-OFF expression system. Supernatants were harvested 4 days post-transfection and subjected to a custom in-house chemiluminometric EIA (EIA-ESLAB). The assay detects HBeAg epitope that gets upregulated upon addition of CpAMs.

HBV DNA quantification in transient transfected cells: HBV DNA levels in Dhsaa-1 transfected supernatants were quantified by real-time quantitative PCR (qPCR) using primers and a probe specific to the HBV core gene.

HBV DNA/pgRNA quantification in PHH: HBV DNA/pgRNA levels were quantified using a TaqMan probe (ABI, Applied Biosystems) specific to the HBV core gene.

HBeAg/HBeAg levels quantification: HBeAg and HBeAg antigen levels were quantified using commercial enzyme-linked immunosorbent assay (ELISA) kits (Affimetrix and Beijing Wensi Biological Pharmacy Enterprise, respectively) as well as in mouse and human ELISAs.

HBV DNA staining: Total HBV DNA levels were visualized using a histochemistry assay (hEMH; KeyGenBioTek) and a set of ICHA probes designed to detect all species of HBV RNA in HepG2 cells. Cells infected with HBV from HepH2 were treated with inhibitors. Four days post-infection, cells were harvested and endogenous RNA was extracted by a high-fidelity EcoRI endoribonuclease (NBS) digestion. High-fidelity EcoRI endoribonuclease (NBS) was then used to generate supercoiled HepG2 cccDNA. EC50 values were calculated using GraphPad Prism software.

CpAMs Impair cccDNA Formation in HepG2-NTCP and PHH Infection Models

### Profiling of CpAMs Antiviral Activities

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<thead>
<tr>
<th>Parameter</th>
<th>HepG2-NTCP EC50 ± SD (nM)</th>
<th>PHH EC50 ± SD (nM)</th>
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<tbody>
<tr>
<td>ENTRK</td>
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### Detection of HBV RNAs by ViewRNA in PHH

- HBRV levels in HBV-infected PHH were detected using ViewRNA. 
- **ETV** showed minimal inhibition of antigen production in PHH when tested at 100 nM (0.5x ETVC50).

### Summary

- Assembly Biosciences has discovered a proprietary series of selective and potent CpAMs.
- CpAMs target core protein and interfere with multiple steps in the viral life cycle.
- CpAMs exhibit broad genotypic activity and retain potency against a nucleos(t)ide resistant variant.
- Treatment with CpAMs early after HBV infection inhibits the formation of cccDNA based on surrogate markers HBeAg, HBsAg and pgRNA levels, and direct cccDNA detection (Southern Blot).
- The suppression of HBV antigens and pgRNA expression is sustainable following CpAM withdrawal.

### CpAMs Represent a novel class of HBV antivirals and Assembly Biosciences' first candidate is progressing into clinical development

References